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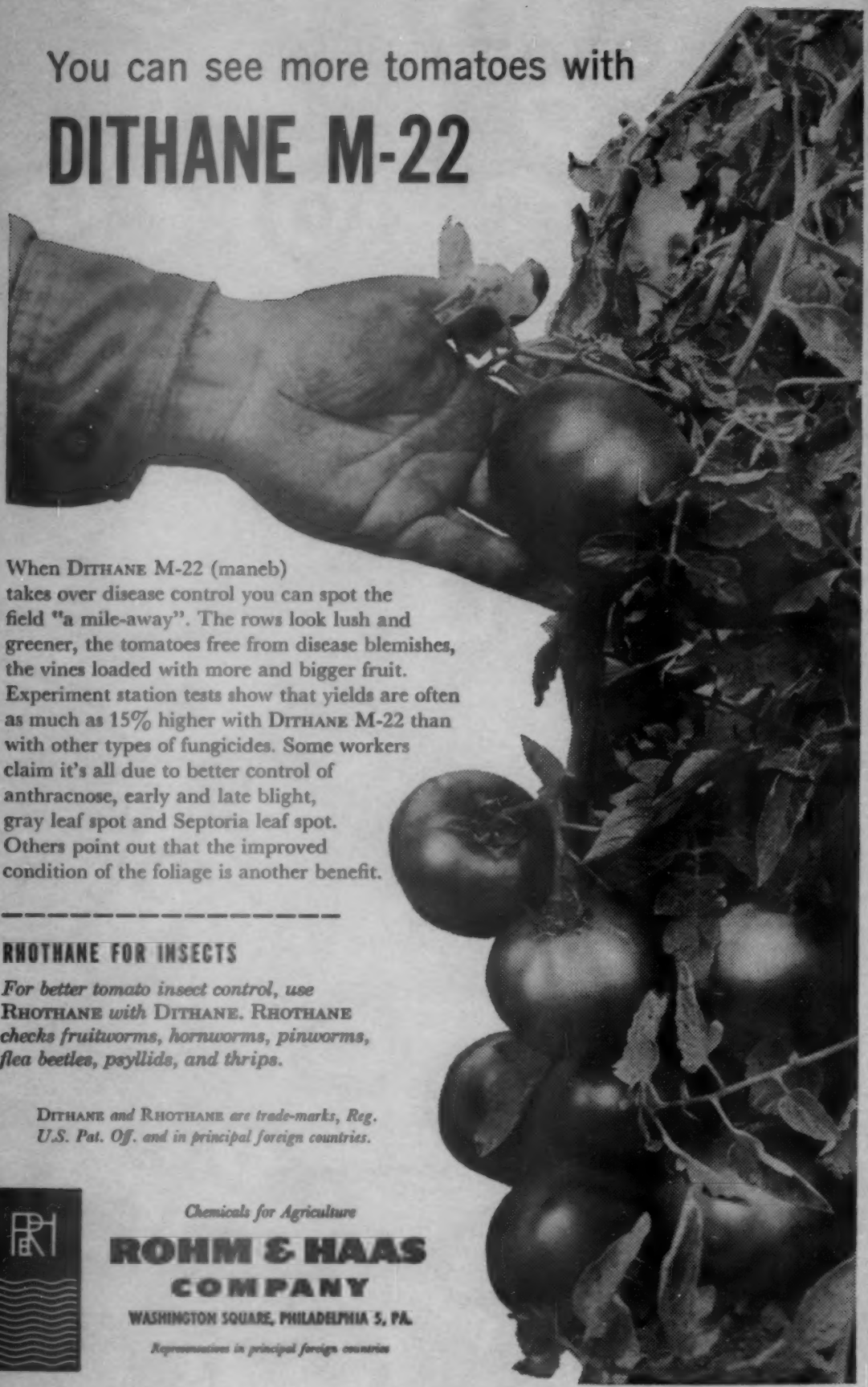

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CARL EDWARD FREDERICK GUTERMAN
1903—1957

L. M. Massey

Carl Edward Frederick Guterman, Director of Research, Director of the Cornell University Agricultural Experiment Station, and Professor of Plant Pathology at Cornell, died in Ithaca on March 27, 1957. In his passing, Cornell University lost an effective and esteemed leader. Those of us who came to know him well lost a friend and valued counselor. To us he was "Gus," a nickname given him by the late Professor H. H. Whetzel and one that he bore throughout his career.

Graduated by the Massachusetts Agricultural College in 1925, Gus came to Cornell to accept a part-time assistantship in the Department of Plant Pathology and to enroll in the Graduate School for an advanced degree. He was granted the degree of Doctor of Philosophy in 1930. On July 1, 1930, he was advanced to Assistant Professor of Plant Pathology and two years later served as Acting Head of the Department. During this early period in his career he showed a marked aptitude both in research and in administration. It was not surprising, then, that with his next appointment, 1933, he was made Assistant to the Director of Experiment Stations. From 1936 to 1942 he served as Assistant Director of the Cornell University Agricultural Experiment Station and Professor of Plant Pathology; from 1942 until the time of his death, he was Director of Research, Director of the Cornell Agricultural Experiment Station, and Professor of Plant Pathology. He was now on his way to a career in administration, in which he was to be very effective. This shifting of activities from plant pathology to administration was recognized by Dr. Guterman and was not passed over lightly, for he was enthusiastic in his interest in plant pathology.

CARL EDWARD FREDERICK GUTERMAN
1903—1957

Dr. Guterman was very conscientious in serving under his appointments and in working to best advantage. He was a tireless worker and continuously sought self-improvement. He was a keen observer, quickly sizing up a situation or problem as to its character and possible solution. He encouraged joint meetings of growers and research workers, a device that did much to assure a full understanding of the nature of problems for which relief was sought. He was called on continuously for advice, cooperation, and assistance by many groups and organizations. Over 500 research projects in agriculture and home economics regularly came under his scrutiny and were his to coordinate. He discharged this task with great ability and took well-merited satisfaction from doing it.

Director Guterman had been a member of the New York State–U.S.D.A. Council since 1948 and was elected chairman in 1954, a member of the New York State Agricultural Mobilization Committee since 1951, and a member of the Cornell Research Foundation since 1950. From 1950 to 1953 he was a member of the Food Protection Committee of the National Research Council. He was a member of the Scientific Advisory Committee of the Boyce Thompson Institute for Plant Research. In the Association of Land Grant Colleges and Universities, Doctor Guterman had been a member of the Experiment Station Legislative Committee since 1951, a member of the Committee on Pesticides since 1952, chairman of the Experiment Station Section and Secretary of the division of agriculture in 1952–53, and a member of the Experiment Station Committee on Organization and Policy from 1942–45. In June, 1955, he was given the Honorary Degree of Doctor of Science by his Alma Mater, the University of Massachusetts.

Gus was easy to work with, because of his outstanding human qualities. He had a keen sense of humor, a ready wit, and an infectious smile. Sincerity and the urge to help were innate qualities that could not be hidden by his modesty. He radiated enthusiasm and kindness and was tolerant and understanding. He was well liked by all as well as respected for his ability. Men of his stature are difficult to replace.

PHYTOPATHOLOGY for November (47: 633-692) was issued December 5, 1957

A STUDY OF TOBACCO MOSAIC VIRUS STRAIN PREDOMINANCE AND AN HYPOTHESIS FOR THE ORIGIN OF SYSTEMIC VIRUS INFECTION [1]

Morris Cohen,[2] Albert Siegel, Milton Zaitlin,[3]
William R. Hudson, and Sam G. Wildman

SUMMARY

Large-scale determinations of virus strain ratios and yields in leaves of *Nicotiana tabacum* L. infected with both the U1 and U2 strains of tobacco mosaic virus (TMV) have been made possible by the electrophoresis of virus isolated ultracentrifugally from leaf extracts. In 18-in. tobacco plants, a tendency towards U1 predominance was highest in young leaves and was not apparent in mature leaves. Mature leaves supported considerable TMV synthesis when inoculated, but such leaves produced little virus when invaded systemically. Young leaves near the apical bud synthesized much virus regardless of the mode of invasion.

In the leaves that were systemically infected subsequent to mixed inoculation, the U1:U2 ratios reached a maximum in those leaves that were in the region of separation from the apical bud at the time of inoculation. The degree of U1 predominance in systemic infection increased with an increase in U1:U2 inoculum ratio; however, at least some U2 was maintained in mixed systemic infections.

Subsequent to mixed inoculation, the systemic symptoms were usually those typical of infection by U1; infrequently, symptoms typical of infection by U2 resulted. The virus extracted from leaves with systemic symptoms of U2 infection was electrophoretically pure U2. In those leaves showing symptoms of U1 infection, the extracted virus was either U1 or a mixture of U1 and U2. In these plants, spotted symptoms, indicative of mixed infection, occurred on some of the lowest systemically infected leaves that showed symptoms. Leaves with symptoms of U1 infection and leaves with spotted symptoms, contained U1 and U2 in widely varying ratios. In a mixed systemic infection of a leaf, therefore, symptoms provide neither a quantitative measure of the relative amounts of each strain present nor an indication of the predominant strain.

The frequency of occurrence of systemic symptoms of U2 infection after mixed inoculation with U1 and U2 decreased with increases of 1 or more of the following: U1:U2 inoculum ratio, inoculum concentration, and number of leaves inoculated. This information is the basis of an hypothesis for the origin of the systemic infection; namely, that systemic infection arises either from the systemic egress of only a limited number of the virus particles present in the inoculated leaf or from the contribution of virus particles from only a limited number of primary infections.

When more than 1 strain of a virus systemically infects the same host plant, one of the strains often predominates. Predominance may be assigned to one of the strains on the basis of symptom expression, of the relative degrees of distribution of the strains throughout the plant, or of the relative amounts of each strain present in any given plant organ or in the plant as a whole.

There seems to be no *a priori* reason for expecting a direct correspondence between symptoms and amounts of each strain in the expression of strain predominance in mixed infections. Nevertheless, in discussing Salaman's experiments (13), involving inoculations of tobacco with a graded series of inocula containing various ratios of the G and S strains of potato virus X, Gäumann (5) considered the resulting spectrum of blended symptoms to reflect the relative degrees of development of each strain in the infected plants. McKinney (11), in contrast, distinguished between these 2 kinds of predominance in his studies of infection of *Nicotiana tabacum* L. by mixtures of tobacco mosaic virus (TMV) strains, employing the terms "dominant symptoms" and "dominant virus." He utilized inoculation tests to determine the dominant virus. McKinney did find a correspondence between dominant symptoms and the dominant virus in the several mixed TMV infections that he studied, but he presented no quantitative data. It is notable that in mixed infections with 2 severe yellow-mosaic-inducing strains derived from common TMV, no symptom dominance occurred, although one of the strains was the dominant virus.

Studies of antagonism or interference between virus strains in the same plant, dependent as they have usually been upon symptom expression or upon identification of the dominant strain, have not led to a clear definition of the process by which 1 strain may become predominant in amount, let alone to a decision as to whether there is a direct or only incidental relationship between dominant symptoms and the dominant virus. Bennett (2), in a review of strain interference, pointed out that "techniques for solving many of the problems involved in interference have been inadequate or lacking."

Foremost among the techniques to be desired in studies of mixed virus strain infections has been a practical one for reliably ascertaining the amounts of

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This work was supported in part by Cancer Research Funds of the University of California, Atomic Energy Commission Contract AT(11-1)-34, Project 8, and U.S.P.H. Grant E-536.

[2] Formerly Fellow of the American Cancer Society, recommended by the Committee on Growth, National Research Council. Now at St. Joseph Hospital, Burbank, California.

[3] Now at Division of Plant Industry, C.S.I.R.O., Canberra, Australia.

each strain present at a given time in a given infected plant organ. Such a technique is offered by the electrophoretic work of Singer et al, (17). They not only demonstrated the presence of 2 strains of TMV, U1 (common) and U2 (a mild strain) (16) in a "common" TMV infection of *N. tabacum* but also determined the relative amounts of each strain present. The applicability of this quantitative electrophoretic technique to problems of mixed infection depends on the use of strains with different electrophoretic mobilities.

We have adopted the electrophoretic technique and employed the U1 and U2 strains of TMV in the present quantitative study of the course by which the U1 strain attains an ascendancy over the U2 strain in *N. tabacum*. This study has been instrumental in leading to experiments on the origin of systemic virus infection. Data from these experiments will be shown to support the hypothesis that a relatively small number of virus units participate in the initiation of systemic infection.

MATERIALS AND METHODS.—Turkish Samsun tobacco (*Nicotiana tabacum* L.) plants were grown in 6-in. pots in the greenhouse. Except where otherwise specified, plants about 18 in. high at the growing point, with leaf development approximately that diagrammatically presented in Figure 1, were inoculated with known artificial mixtures of centrifugally purified U1 and U2 strains of TMV. One to 4 successive leaves per plant were inoculated at various levels; i.e. leaves about 1 in. in length to mature leaves over 7 in. in length. Comparable leaves on all plants in an experiment were assigned corresponding numbers in accordance with the plan illustrated by Figure 1. By this system, the youngest set of leaves inoculated in an experiment might, for example, be leaves 15 through 17.

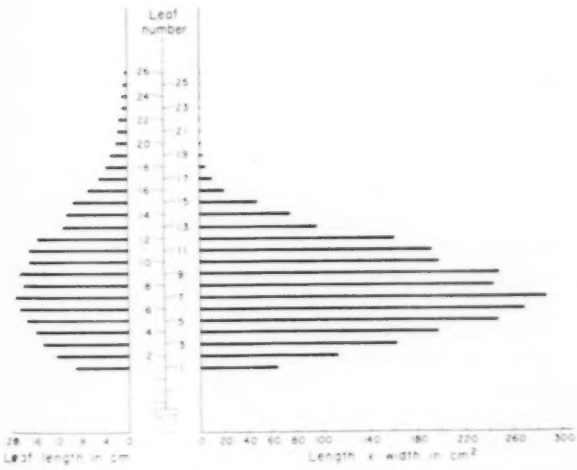

FIG. 1. Diagrammatic representation of the assignment of leaf numbers in the 18-in. plants used in most of the experiments. Leaf lengths and relative leaf areas are given as bar graphs. At the time of inoculation, the flower primordia had begun to form. The smallest leaves at the top of the plant are not shown. Leaf 1 was usually the fifth to the seventh leaf above soil level.

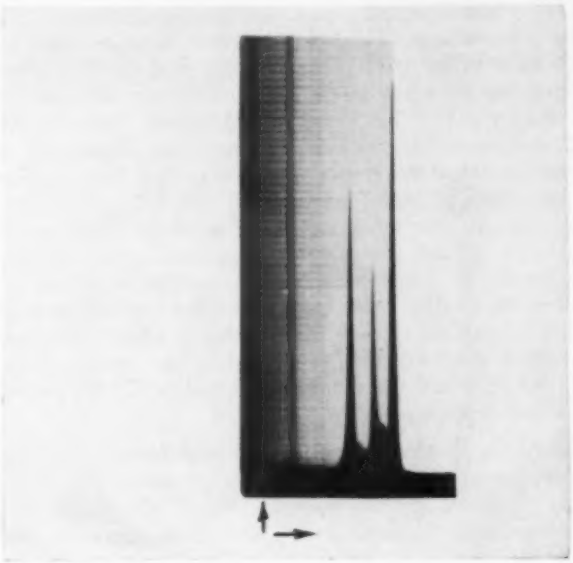

FIG. 2. Electrophoretic pattern of virus ultracentrifugally isolated from leaves of *Nicotiana tabacum* infected with both the U1 and U2 strains of tobacco mosaic virus. The vertical arrow indicates the starting boundary, the horizontal arrow the direction of migration. The leading peak represents U1, the trailing peak U2, and the central peak an aggregate of both strains.

Leaves were harvested periodically, usually as combined samples from 2 or more plants; the midribs were removed and the laminae stored at —22°C prior to extraction of the virus for strain analysis. The frozen leaves were homogenized in cacodylic acid buffer (0.08 M NaCl, 0.02 M Na cacodylate, 0.0033 M cacodylic acid; pH 6.9) in a Waring Blendor. The brei was centrifuged for 20 minutes in a model SS1 Servall centrifuge at a Variac setting of 75. Virus was purified from the supernatant by 2 cycles of high-speed (30,000 rpm, 1 hour) and low-speed (10,000 rpm, 20 minutes) centrifugation in the No. 30 rotor in the model L Spinco ultracentrifuge. The virus pellets from the high-speed centrifugation were all resuspended in the cacodylic acid buffer.

The amount of virus present in each sample was determined by multiplying the nitrogen content, as determined by Nesslerization (10), by 6.25.

The relative amounts of U1 and U2 present in each virus sample were determined electrophoretically in an apparatus designed and built by Frank Pearson Associates. Most samples were run for 40 minutes at a potential gradient of 5.6 volts/cm^2; however, with less than 0.6 mg virus/ml (down to the 0.1 mg/ml minimum that could be analyzed), samples were run for only 20 minutes to minimize diffusion of the boundaries. A period of 20 minutes was sufficient for the resolution of the electrophoretic components.

Electrophoretic patterns of virus centrifugally iso-

lated from infected leaves containing both U1 and U2 display 3 peaks (Figure 2), as has been reported by Singer et al (17). The leading and trailing components have the same electrophoretic mobilities as those reported by Siegel and Wildman (16) for the pure strains U1 and U2, respectively. Singer et al suggest that the central peak is a centrifuge artifact consisting of equal parts of U1 and U2, and experiments in this laboratory with artificial mixtures of the 2 strains strongly support this notion. Consequently, in our electrophoretic analysis of mixed infections, half of the area under the central peak is added to that of each of the other 2 peaks. The corrected areas are then converted to percentage of the total area and the results expressed as U1:U2 ratios. Examples are given in Table 1.

Although there is a negligible difference between duplicate electrophoretic determinations of strain ratios in a mixture, certain errors inherent in the electrophoretic method (1) lead to an overestimation of the amount of the leading component in a multiple-peak system. The overestimation reaches a maximum of about 15 per cent with artificial 50:50 mixture of the 2 strains. This bias is of the same order regardless of whether or not the preparation presents a 2-component pattern or a 3-component pattern. Data reported in this paper were not corrected for the bias.

RESULTS.—*Mixed infections in inoculated leaves.*—Since systemic symptoms characteristic of those induced by U1 generally result from mixed inoculation with U1 and U2, it was of interest to investigate the course of mixed infection in the inoculated leaves for its possible bearing on the nature of the systemic infection to which it gives rise. Symptoms of infection in inoculated leaves appear late in the infection process and generally consist of diffused chlorotic spots, which yield no information about the amounts of U1 and U2 present in those leaves.

TABLE 1.—*Electrophoretic pattern of artificial and natural mixtures of the U1 and U2 strains of tobacco mosaic virus subjected to various ultracentrifugal treatments*

Source of U1 and U2 mixture	Virus concentration in mg/ml	Percentage of total area under indicated peak			Interpreted U1:U2 ratio
		Fast (U1)	Middle	Slow (U2)	
Artificial 40:60 mixture of purified U1 and U2	6.0	43	0	57	43:57
Same after 2-cycle differential ultracentrifugation	6.0	37	13	50	44:56
Extract of mixedly infected leaves after slow-speed centrifugation	2.5	43	0	57	43:57
Same after 2-cycle differential ultracentrifugation	5.9	38	15	47	46:54

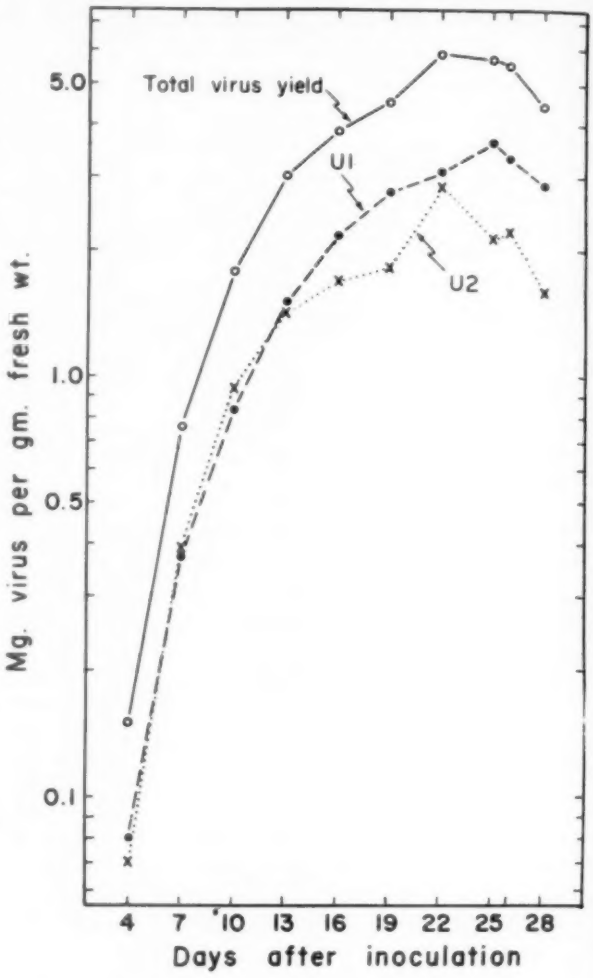

FIG. 3. Amounts of U1 and U2 strains of tobacco mosaic virus in inoculated mature leaves at various times after inoculation. U1 showed a slight predominance in rate of increase after the 13th day after inoculation. Inoculated leaves were dead on the 28th day after inoculation.

1) *Little or no tendency towards U1 predominance in inoculated mature leaves.*—When fully expanded leaves were inoculated with mixtures of U1 and U2 in ratios of 20:80, 50:50, and 80:20, electrophoretic analyses showed that the ratios of U1 to U2 developed in those leaves were not far removed from the original inoculum ratios. With the 20:80 inoculum ratio, no significant differences have been observed between inoculum ratio and ratios developed in inoculated leaves. With 50:50 and 80:20 U1:U2 inoculations, however, a slight increase in U1:U2 ratio in inoculated leaves has been observed, although the time at which this increase has become manifest has varied from experiment to experiment. An example of such slight U1 predominance is shown in Figure 3, which illustrates the time course of strain synthesis following a 50:50 inoculation. The rates of synthesis were

approximately the same from the fourth to the thirteenth day of infection; thereafter the increase of U1 was somewhat greater than that of U2.

2) *U1 predominance in inoculated young leaves.*—In contrast to the results with mature leaves, young leaves that had been inoculated with a mixture of the 2 strains consistently yielded virus with a higher U1:U2 ratio than that of the inoculum. Single leaves varying in development from mature to very young (leaves 9, 13, 17, 21, and 25, Figure 1) were inoculated, 1 leaf per plant, with a 50:50 mixture of U1 and U2. The 28-day U1:U2 ratio developing in these leaves was higher in the younger than in the older leaves, reaching a maximum (100% U1) in a leaf (leaf 25) that was about ½ in. long at the time of inoculation. The lowest U1:U2 ratios developed were 59:41 in leaf 9 and 48:52 in leaf 13. Intermediate U1:U2 ratios, ranging from 65:35 to 81:19, occurred in leaves 17 and 21.

Similarly, when groups of 3 mature leaves (leaves 3 to 5) or 3 young leaves (leaves 15 to 17) were inoculated with a 50:50 inoculum, the U1:U2 ratios developed in the younger leaves were higher than in the older leaves from the eighth to the 21st day of infection. During this period, however, both strains increased at about the same rate in the younger group of leaves. In order to account for this observation, one can postulate the operation of 1 or more of the following possibilities: 1) more U1 than U2 primary infections may have been initiated in the younger leaves; 2) U1 may have been more widely distributed than was U2 from the original sites of infection to other parts of the inoculated young leaves during the first 8 days of infection; 3) U1 synthesis per primary infection may have been greater than that of U2 during a restricted period while the leaf was still actively expanding. Any of these postulates can then be combined with the notion that, with nearly complete leaf expansion by the eighth day of infection, conditions for strain synthesis approximated those of mature leaves, which support equal rates of U1 and U2 synthesis during the first 1 or 2 weeks after inoculation.

Mixed infection in systemically invaded leaves.—Whatever may lie at the basis for pronounced U1 predominance in young inoculated leaves, no clues emerge from these studies to explain the usual identical systemic mosaic symptoms, characteristic of infection by U1, that occur regardless of whether mature or young leaves are inoculated with the mixed inocula we have employed. If the nature of mixed infections in inoculated leaves does play a determining role in the systemic infection, the first step towards the elucidation of that role might be an analysis of the mixed systemic infection.

1) *Virus yields in inoculated and in systemically infected leaves.*—When directly inoculated, all leaves of a tobacco plant from a mature leaf (leaf 9) upward to a young leaf (leaf 25) supported considerable virus synthesis (Table 2). In contrast, when

TABLE 2.—*Total virus yields from inoculated and noninoculated tobacco leaves at comparable leaf levels 28 days after inoculation of 1 leaf per plant with a mixture (5×10^{-3} mg/ml) of the U1 and U2 strains of tobacco mosaic virus*

Leaf number[a]	Mg virus per leaf	
	Directly inoculated leaves	Systemically infected leaves
9[b]	17.7	17.7[c]
	20.0	
13	27.5	[d]
	36.0	
17	20.0	[d]
	39.5	
21	22.4	0.18
	14.4	
25	26.9	13.2
29		6.1
33		4.1

[a] Leaves numbered from bottom to top. See Figure 1.
[b] Leaf 9 died between the 26th and 28th day after inoculation.
[c] First inoculated leaf 9 shown in second column.
[d] No visible virus pellet obtained upon ultracentrifugation of leaf extract.

comparable leaves were systemically invaded as the result of the inoculation of only leaf 9, all of them above leaf 9, excepting those that were near the growing point at the time of inoculation, produced relatively little virus in 1 month's time. High virus yields in noninoculated leaves were obtained only in the region of leaf 25 and upward (Table 2). Thus, there is a group of mature and expanding leaves above an inoculated mature leaf in which very little virus is produced. Similar observations have been reported by Holmes (6), Samuel (15), and Sadasivan (12). The explanation for the higher yields in noninoculated young leaves than in noninoculated older leaves apparently lies in the rapid, thorough spread of systemically invading virus through the young leaves (7, 14, 15). In contrast, in older leaves, spread of the virus from a limited number of infectious centers established by systemic invasion is slow (15). The high virus yields in directly inoculated mature leaves seems, therefore, to derive from the initial distribution of many infectious centers throughout the leaf.

From the experiment just described, it would be expected that if young leaves were inoculated, the "gap" in virus synthesis in leaves situated just above the inoculated leaf would not occur. Indeed, when young leaves near the terminal bud were inoculated, all noninoculated leaves situated still higher on the plant produced considerable virus (Table 3).

2) *Symptoms vs. strain ratios in systemic infections.*—Mosaic symptoms are usually restricted to those leaves that were near or in the terminal bud at the time of inoculation. Inoculations with mixtures of U1 and U2 have generally led to systemic symptoms characteristic of those induced by U1 (hereafter referred to as U1 symptoms).

There is an additional systemic symptom that per-

TABLE 3.—*Total virus yields from tobacco leaves at various leaf levels 12 days after inoculation of either 3 mature or 3 expanding leaves per plant with a 50:50 mixture (2×10^{-3} mg/ml) of the U1 and U2 strains of tobacco mosaic virus*

Leaves[a] assayed as combined sample	Mg virus per leaf[b] when indicated leaves were inoculated	
	Leaves 3–5 (mature)	Leaves 15–17 (expanding)
3–5	(6.3) (3.8)	
6–8	0.09	
9–11	0.15	
12–14	0.08	
15–17	0.11	(1.5) (1.8)
18–20	1.4	3.5
21–23	1.9	3.4
24–26	0.9	1.4

[a] Leaves numbered from bottom to top. See Figure 1.
[b] Values in parentheses are for inoculated leaves; all others are for systemically invaded leaves.

mits the recognition of at least some mixed systemic infections, for it has been observed only after mixed inoculations. This is the "spotting" reported by Holmes (8). It consists of a dense distribution of specks of normal green on a light green background, in contrast to the light-, normal-, and dark-green mosaic typical of U1 symptoms. Spotting may cover the whole surface of a leaf or only a part, the remainder showing either no symptoms or U1 symptoms. Spotting is usually restricted to 1–3 of the lowermost leaves exhibiting systemic symptoms.

The U1:U2 ratio of virus isolated from spotted leaves has been found to fall between 37:63 and 89:11. In the leaves at higher levels in the plant, which display typical U1 symptoms, more U1 than U2 has usually been found to be present; however, the U1:U2 ratios in these leaves have ranged from 100:0 down to 27:73. This range includes within it the U1:U2 ratios found in spotted leaves; therefore, it seems likely that spotting is a response of leaves to mixed systemic invasion that depends more on the physiological condition of the leaves than upon any precise U1:U2 ratio.

Infrequently, symptoms characteristic of those induced by U2 (hereafter referred to as U2 symptoms) appeared after mixed inoculation; in this event, these symptoms developed in every leaf exhibiting systemic symptoms. Such leaves were found to contain only the U2 strain.

From our observations of both strain ratios and systemic symptoms in the same leaves, we must conclude that, with the exception of U2 symptoms, symptoms in a leaf not only fail to provide a quantitative measure of the strain ratio in that leaf but are also not useful in determining which of the strains, U1 or U2, is present in the greater amount (cf. 11).

3) *U1 predominance in systemically infected leaves.*—Although the U1:U2 ratio in mature leaves inoculated with a 50:50 mixture of the strains may be slightly higher than 50:50, in our experience it has not exceeded 70:30. In contrast, the ratio can be as high as 100:0 in systemically infected leaves of these same plants.

The experiment summarized in Table 4 is an ex-

TABLE 4.—*Percentage of total virus found to be strain U1 in systemically invaded tobacco leaves 12 and 21 days after 3 mature or 3 expanding leaves per plant were inoculated with various mixtures (10^{-3} mg/ml) of the U1 and U2 strains of tobacco mosaic virus*

Leaves[a] assayed as combined sample	Percentage of total virus found to be U1 in systemically infected leaves[b] 12 and 21 days after inoculation of indicated leaves with inocula with indicated U1:U2 ratio																	
	Leaves 3–5 (mature) inoculated												Leaves 15–17 (expanding) inoculated					
	100:0		80:20		50:50		50:50[c]		20:80		0:100		100:0		50:50[c]		0:100	
	12 Days	21 Days	12 Days	21 Days	12 Days	21 Days	12 Days	21 Days	12 Days	21 Days	12 Days	21 Days	12 Days	21 Days	12 Days	21 Days	12 Days	21 Days
3–5	(100) (100)		(92) (83)	(91)	(59) (62)		(62) (62)		(20) (20)	(17)	(0)							
15–17	*	100	*	*	*	61 69	*		*	50	*		(100)	(100)	(62) (85)	(62) (75)	(0)	(0)
18–20[d]	100	100	100	100	96	78 80	98	98	51	62	0		100	100	90	80		
21–23[d]	100	100	100	100	96	71 85	100	99	65	54	0		100	100	92	85	0	
24–26	100	100	100	100	85	63 66	83	97	40	36	0		100	100	78	80		
27–29	*	100	*	97	*	64 79	*	96	*	27	*	0	*	100	*	72	*	0

[a] Leaves numbered from bottom to top. See Figure 1.
[b] Figures in parentheses are for inoculated leaves; all others are for systemically invaded leaves.
[c] Inoculum concentration was 2×10^{-3} mg/ml in these tests and 10^{-3} mg/ml in all others.
[d] Leaves that were near the point of separation from the apical bud at the time of inoculation. In general, maximum systemic U1:U2 ratios occurred in this group of leaves.
* Yield of virus too low to permit electrophoretic assay. Yields from leaves 6–17 were also too low.

ample of U1 predominance in mixed systemic infection. Mature leaves and young expanding leaves were inoculated with various mixtures of the strains. The virus preparations isolated from inoculated and from systemically invaded leaves, harvested on the 12th and 21st day after inoculation, were analyzed for their content of U1 and U2. When mature leaves were inoculated, the subsequent U1:U2 ratios in the systemically infected leaves were always higher than those in the inoculated leaves. When young leaves were inoculated, the subsequent U1:U2 ratios in younger noninoculated leaves were generally at least as high or higher than in inoculated leaves. With an increase in U1:U2 inoculum ratio, the tendency towards U1 predominance in the systemically infected leaves increased. When the inoculum ratio was sufficiently low, as with the 20:80 ratio, U2 instead of U1 predominated in some of the systemically infected sets of leaves; however, even in these leaves, the tendency towards U1 predominance was apparent in that the U1:U2 ratios in those leaves exceeded the ratio developed in the inoculated leaves.

Regardless of whether mature or young expanding leaves were inoculated, U1 predominance tended to reach a maximum in those very young systemically invaded leaves that had been near the point of separation from the apical bud at the time of inoculation (leaves in the region of levels 18–23). It should be recalled, in this connection, that a striking U1 predominance also occurred in young leaves that were directly inoculated with a 50:50 inoculum. It seems, therefore, that predominance will be accentuated in a particular group of leaves situated in the apical region at the time of inoculation of a plant, regardless of whether those leaves are directly inoculated or systemically invaded.

It should also be recalled that when young leaves were mixedly inoculated, the rates of increase of U1 and U2 were about the same by the time measurements were made and that, nevertheless, U1 predominance had already been established. A similar phenomenon was found in systemically invaded leaves that were near to or within the apical bud at the time of inoculation of mature leaves. Regardless, then, of the pathway of viral invasion, the tendency towards U1 predominance was established early during the infection of very young leaves.

In a few instances, when U1:U2 inoculum ratios up to 99:1 were employed, ratios as low as 56:44 were observed in some systemically infected leaves. Thus, although there was a tendency towards U1 predominance, there was at the same time a tendency towards maintenance of U2 in the mixed infection. This may have survival value for the U2 strain. For example, when U2 was first isolated as an electrophoretic fraction from a repeated greenhouse culture of "common" TMV, it constituted about 10 per cent of the total virus present in infected leaves; the remainder corresponded to the U1 strain (17). Bennett (2) has pointed out that certain strains may "reach an equilibrium" in the same host plant.

AN HYPOTHESIS FOR THE ORIGIN OF SYSTEMIC INFECTION.—The U1:U2 ratios in mixedly inoculated mature leaves approximate or only slightly exceed those of the inocula. In such plants, a systemic U1 predominance, accompanied by U1 symptoms in the younger systemically infected leaves, generally results. In a few such plants, however, U2 symptoms have been observed, and this has always been associated with the exclusive development of U2 in systemically infected leaves. In 1 striking instance, U1 symptoms appeared in 1 plant and U2 symptoms in another, although 1 mature leaf on each plant had been inoculated with the same 50:50 inoculum. Both inoculated leaves, furthermore, developed about the same U1:U2 ratio—60:40.

This phenomenon could be explained if the systemic invasion of the plant were to be initiated by only a few of the many virus units present in the inoculated leaf. Then, following a mixed inoculation, such virus units should more or less frequently be all of 1 strain. This line of reasoning led to the formulation and experimental testing of an hypothesis regarding the composition of the "systemic inoculum" that issues from inoculated mature leaves.

Formulation of the hypothesis.—The hypothesis presented here is that a limited small number of the large population of virus units in the inoculated mature leaf participate in a random fashion in establishing the systemic infection. The following considerations and assumptions have entered into our formulation of this hypothesis.

1) When a mature leaf is inoculated with a mixture of U1 and U2, it is assumed that primary infections will be randomly distributed without regard to virus strain.

2) Virus units of each strain in the inoculated leaf are assumed to participate nonselectively in the initiation of systemic infection.

3) The U1:U2 ratio in the inoculated mature leaf at the time of initiation of systemic infection is approximately that in the original inoculum. This is justified by the following: a) systemic egress of infective particles from the inoculated mature leaf under our conditions occurs some 4–8 days after inoculation, as indicated by an experiment (unpublished) in which inoculated leaves were detached at different times after inoculation, and b) during this period from the fourth to the eighth day of infection, the U1:U2 ratio in inoculated mature leaves approximates that in the inoculum.

4) When U2 symptoms appear subsequent to mixed inoculation of a mature leaf, the "systemic inoculum" is assumed to have consisted entirely of U2. The justification for this is the observation that only U2 is electrophoretically detectable in the systemically infected leaves of these plants. In contrast, when U1 symptoms appear, then U1 alone or both U1 and U2 are electrophoretically detectable in the systemically infected leaves.

The foregoing considerations lead to a mathematical expression for the frequency with which systemic U2 symptoms could be expected to appear after mixed inoculation of mature leaves. If we consider the binomial expansion of $(p + q)^m$, where p and q are the fractions of U1 and U2, respectively, in the virus in the inoculated leaf from which the "systemic inoculum" is drawn, and m is the number of virus units in the inoculated leaf that initiate the systemic infection, then the term q^m represents the frequency with which the "systemic inoculum" will consist of virus units of the U2 strain only.

Test of the hypothesis.—With q set equal to the fraction of U2 in the inoculum applied to a mature leaf, a value for m can be found such that q^m will approximate the observed frequency of U2 symptoms. This has been done in the experiment summarized in Table 5. Inoculum mixtures containing various U1:U2 ratios, the total inoculum concentration being kept constant, were applied to 1 mature leaf on each of 100 plants per treatment. The plants were about 1 ft. tall at the time of inoculation and were grown in 4-in. pots. The q values in Table 5 are the fractions of U2 in each inoculum. Values of m were calculated from the fractions of plants exhibiting U2 symptoms. These values vary about 2-fold in range within an experiment. It is striking that all calculated values of m are small. It is possible to select a single value of m for the experiment that will permit calculation of values of q^m whose differences from observed frequencies of U2 symptoms are a minimum. Such values of q^m, with m equal to 10, appear in the third column of Table 5. There is good agreement between observed and predicted frequencies of U2 symptoms. This supports the hypothesis that the number of virus units that initiate systemic infection is small. Furthermore, such agreement suggests that the possible kinds of "systemic inocula" that lead to U1 symptoms also occur with frequencies predicted by the successive terms of the binomial expansion of $(p + q)^m$. Presumptive evidence for this might be provided by a correspondence between predicted frequencies, p^m, of systemic infection by pure U1 and the frequency of occurrence of U1 symptoms without the spotted symptoms indicative of mixed systemic infection.

TABLE 5.—*Observed and predicted frequencies of U2 symptoms following inoculation of 1 leaf per tobacco plant, 100 plants per treatment, with various mixtures (1.8×10^{-2} mg/ml) of the U1 and U2 strains of tobacco mosaic virus, together with the calculated number of virus units (m) in the "systemic inoculum"*

Proportion of U2 in mixed inoculum (q)	Frequency of U2 symptoms		Calculated no. of virus units[b] per inoculated leaf providing the "systemic inoculum" (m)
	Observed	Predicted[a] (q^m)	
0.80	0.05	0.11	13
0.85	0.24	0.20	9
0.95	0.59	0.60	10
0.99	0.80	0.90	22

[a] Predicted from the q^m term of the binomial expansion of $(p + q)^m$, where p and q are the proportions of U1 and U2 in the virus pool from which the "systemic inoculum" is drawn, assumed equal to the proportions of U1 and U2 in the inocula, and m, the number of virus units per inoculated leaf providing the "systemic inoculum," is assumed to equal 10.

[b] Calculated $m = \frac{\log \text{observed frequency of U2 symptoms}}{\log q}$, where the proportions of U2 in the mixed inocula are assumed to be q.

If the hypothesis is valid, then it follows that the frequency of pure systemic infection by U2 should be less when more than 1 mature leaf per plant is inoculated with a mixture of U1 and U2 than when only 1 leaf per plant is so inoculated. A preliminary experiment (unpublished) has provided such a result.

The virus unit.—The term "virus unit," as we have used it, cannot yet be defined. We cannot distinguish between the possibilities that in the inoculated leaf m may represent a small number of infectious particles, which initiate the systemic infection, or only some of the primary infections, each of which can contribute 1 or more infectious particles to the systemic infection. The latter alternative may be subject to experimental visualization. Experiments might be conducted in which, for each U1:U2 inoculum ratio, the inoculum concentration is varied and observations made, via the starch-lesion method of Holmes (6), to see whether there is an equivalence between calculated m values and the average number of veinal starch-streaks per leaf. The notion proposed here is that those starch-lesions that are confluent with starch-streaks may constitute the primary sources of "systemic inoculum."

Factors influencing m and q.—Is m, the number of virus units that initiate the systemic infection, relatively constant, or is it a function of the total number of virus units present in the leaf? In the attempt to answer this question, a single U1:U2 inoculum ratio (5:95) was applied in different total inoculum concentrations to 1 mature leaf on each of 80 1-ft. plants per treatment. The observed frequencies of U2 symptoms (Table 6) decreased with increasing inoculum concentration. The calculated values of m that would allow the prediction of those frequencies are also tabulated. With an increase in inoculum concentration, there appears to have been an increase in the number of virus units initiating the systemic infection.

An alternative interpretation of the results in Tables 5 and 6 may be made if either or both of the first 2 assumptions on which the formulation of the hypothesis was based are untrue. If, contrary to the first assumption, strain-selective exclusion were to operate during inoculation of mature leaves with mixtures of U1 and U2, then an increase in inoculum concentration should affect both p and q. As an example, if a degree of exclusion of U2 by U1 had occurred in

TABLE 6.—*Frequency of occurrence of systemic U2 symptoms after inoculation of 80 tobacco plants per treatment with different concentrations of a 5:95 mixture of the U1 and U2 strains of tobacco mosaic virus, together with the calculated number of virus units (m) in the "systemic inoculum" and, assuming selective exclusion at inoculation and a constant m, calculated proportions (q) of the U2 strain in the virus pool from which the "systemic inoculum" was drawn*

Total inoculum concentration (mg/ml)	Observed frequency of U2 symptoms	Calculated no. of virus units[a] per inoculated leaf providing the "systemic inoculum" (m)	Calculated[b] values of q with m = 13.5
$\times 10^{-3}$			
4	0.50	13.5	0.95
20	0.175	34	0.88
100	0.075	50.5	0.83
500	0		

[a] Calculated $m = \frac{\log \text{observed frequency of U2 symptoms}}{\log q}$,

where 0.95, the proportion of U2 in the mixed inoculum, is assumed to be equal to q.

[b] Calculated $q = \text{antilog}\left(\frac{\log \text{observed frequency of U2 symptoms}}{m}\right)$.

the experiment summarized in Table 6, then q would have decreased with increasing inoculum concentration. Values of q (last column of Table 6), calculated from the data on the assumption of a constant m of 13.5, do decrease with increase in the inoculum concentration. Only a slight decrease in q could have accounted for the great decrease in frequency of U2 symptoms observed.

A mechanism whereby the systemic egress of the strains is to a degree selective would negate the second assumption. This would involve a "systemic inoculum" in which the U1:U2 ratio was higher or lower than that of the virus pool in inoculated mature leaves from which the "systemic inoculum" was derived. For the experiment in Table 5, however, it can be shown by calculation that, with appropriate adjustments in m, the efficiency with which U2 in the inoculated leaf contributed to the "systemic inoculum" could have ranged from one-fifth indefinitely upwards of that of the U1 strain without introducing any experimentally significant differences in the predicted frequencies of systemic U2 infection. To obtain the differences necessary for ascertaining the degree of selective systemic egress of the strains from an inoculated mature leaf would require inoculum mixtures leading to the development of a 50:50 U1:U2 ratio in the virus in the inoculated leaf at the time the "systemic inoculum" is constituted. Unfortunately, such an experiment is made impractical by the large number of plants it would require.

For each series of mixed inoculations of fixed inoculum concentration, the consequences of these modified interpretations do not prevent the selection of a rational value for m and a single correction factor for all values of q, from which frequencies of systemic infection by pure U2 can be predicted. It should be possible to ascertain whether or not selective exclusion at inoculation occurs, and this may permit the establishment of the kind of relationship that exists between m and the inoculum concentration.

Significance of the "systemic inoculum" in establishing systemic infection.—An important question concerning the role played by a given "systemic inoculum" from an inoculated mature leaf in establishing a mixed systemic infection is raised by the early tendency towards U1 predominance in the mixed systemic infection of young leaves. In our experiments, exclusion effects at inoculation need not be considered because of the relatively low inoculum concentrations. Furthermore, the U1:U2 ratio of the virus pool in the inoculated mature leaf from which the "systemic inoculum" is drawn approximates the original inoculum ratio and so does not help to explain the phenomenon of early systemic U1 predominance in young leaves. Alternative possibilities are the following: 1) a greater efficiency of systemic egress of U1 than of U2 from the inoculated leaf; 2) a more extensive systemic distribution of U1 than of U2 to young leaves; 3) a greater synthesis of U1 than of U2 during systemic transit of the virus strains; and 4) a greater interveinal invasiveness of U1 than of U2 in the systemically infected leaves. No information with regard to the first 2 alternatives is available. The third is probably a negligible factor in the phenomenon in view of the occurrence of virus-free stem regions through which the virus is known to have passed (4, 8, 9). There is a basis for the fourth possibility in the work of Holmes (8) with his common, mottling, and masked strains of TMV. He demonstrated that the extent of viral invasion of the veins of young leaves at the time of vein clearing is about the same for these 3 strains, but that the relative invasiveness and extent to which infective centers are subsequently established in the laminae is in the following order: common > mottling > masked. Holmes had evidence that the ability of virus in the veins of a systemically invaded leaf to spread into the interveinal tissue may be related to the degree of maturity of the different parts of a growing leaf.

After the mixed inoculation of young leaves, there is in systemically infected younger leaves a similar early tendency towards a higher U1:U2 ratio than that in the original inoculum. This might be explained in part by the early increase of the U1:U2 ratio in the inoculated young leaf, thus permitting a greater contribution of U1 to the "systemic inoculum" than would be expected from the ratio in the original inoculum.

DISCUSSION.—The study of virus strain predominance, which formerly concerned itself merely with leaf symptoms and determination of the dominant strain by subinoculation, can now be placed on a quantitative basis by means of the electrophoretic

method of assaying virus strain mixtures.

During our studies, however, there have arisen questions that still remain to be answered if the full significance of some of our findings is to be realized, although they have aided our thinking about the overall problem. In this category are such questions as the following: 1) Does exclusion of 1 strain by another occur upon mixed inoculation? 2) What are the rates of virus strain synthesis in the inoculated leaf between the time of inoculation and the time when there is sufficient virus for electrophoretic analysis? 3) What are the relative rates of spread in the mixedly inoculated leaf of primary infections of each strain, and to what extent are subcenters of infection established? 4) How do inoculum ratio and concentration, early rates of virus strain spread and of synthesis in the inoculated leaf, and relative tendencies of the strains towards egress from the inoculated leaf affect the constitution of the "systemic inoculum"? 5) What is the pattern of mixed infection in systemically invaded leaves?

Insight into most of these questions will probably depend not only on locating the infectious centers but also on their identification with respect to virus strain. Two approaches can be suggested. By one, starch lesions of each strain in an inoculated leaf can be identified by subinoculation. This experiment is feasible since we have determined that tissue from starch lesions is infectious and that tissue from between starch lesions is noninfectious. Also, it might be possible to locate infective centers and identify the infecting strain by applying fluorescent antibodies (3) specific for each strain to filter papers between which an infected leaf has been pressed.

The problem concerned with the possible selective systemic egress of the strains from the mixedly inoculated leaf might be amenable to the following attack. We have already noted Holmes' (8) observation of a differential invasiveness of strains into interveinal tissue of systemically infected leaves. This phenomenon could be investigated by means of electrophoretic analysis of the virus in midrib tissue relative to that in the remaining tissue of a systemically infected leaf. The U1:U2 ratio in the midrib would, however, also be of significance relative to the U1:U2 ratio in the inoculated leaf of the same plant. If the distributional mode of the U1:U2 ratios in the systemically infected leaf midribs of a plant were either to approximate or stand in a particular relation to the ratio in the inoculated leaf of that plant, then an effective degree of selective egress of the strains from the inoculated leaf might be calculated. With equal ratios it would be reasonable to infer that no strain-selective egress from the inoculated leaf had occurred. This information could not only be used in predicting the frequency of occurrence of pure U2 systemic infection, in accordance with the hypothesis for the origin of systemic infection, but also in evaluating the number of virus units providing the "systemic inoculum."

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A DISEASE OF ROSES CAUSED BY A ROOT-LESION NEMATODE, PRATYLENCHUS VULNUS[1]

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SUMMARY

A disease of roses has been demonstrated to be caused by a root-lesion nematode *Pratylenchus vulnus* Allen & Jensen. Root-lesion nematode disease is characterized by stunted chlorotic plants with necrotic root systems that are smaller than normal. The disease was reproduced by growing rose cuttings in field soil in the greenhouse, and it was eliminated by fumigation of the soil prior to planting. Infestation of soil with *P. vulnus* caused the disease. *Trichodorus christiei* Allen from rose fields did not produce this disease. Leaves from plants infested with *P. vulnus* were lower in iron, copper, and potassium than were control plant leaves.

Pratylenchus pratensis (de Man) Filipjev, a root-lesion nematode, was first reported from rose roots in 1937 (3). In 1953, *P. vulnus* Allen & Jensen was recorded from rose roots in California (10). The association of root-lesion nematodes with disease of roses was first recorded by Schindler (8) and Oostenbrink (7) in 1956. Schindler reported the genus *Pratylenchus* to be the most prevalent nematode genus in 34 commercial rose greenhouses in the United States; in general, affected plants were showing chlorosis and poor growth. *P. pratensis* and *P. penetrans* (Cobb) Sher & Allen were reported by Oostenbrink (7) from *Rosa canina* L. associated with a disease in the Netherlands. *P. scribneri* Steiner has been reported from rose roots in a commercial greenhouse in California (6). These plants exhibited chlorosis and were stunted.[2]

Pratylenchus vulnus (2) and *Trichodorus christiei* Allen (1) are widely distributed in southern California on field-grown roses. Chlorotic stunted plants with necrotic root systems have been found associated with the presence of large numbers of *P. vulnus* in the roots and of *T. christiei* in the surrounding soil. Field fumigation with various soil fumigants before planting (9) has resulted in increased growth of roses.

Because of the importance of root-lesion and stubby-root nematodes in other diseases (5, 11), tests were undertaken to ascertain whether these nematodes are a factor in rose production. Two controlled tests were conducted in the greenhouse: 1) fumigation of field soil with various fumigants and 2) infestation of soil with suspected nematodes.

SOIL-FUMIGATION EXPERIMENT.—*Materials and methods.*—Soil from a rose field in Ontario, California, containing *Pratylenchus vulnus* and *Trichodorus christiei* was used to fill 24 3-gal. glazed crocks, which were divided into 4 series of 6 crocks each. Crocks of 3 series were treated with fumigants: the first series with Dowfume W-85 (1,2-dibromoethane), 1 ml per crock; the second series with D-D (1,3-dichloropropene; 1,2-dichloropropane), 3 ml per crock; and the third series with chloropicrin (trichloronitromethane), 3 ml per crock. The fumigants were pipetted into the center of the crock, and the soil was tamped down lightly. A ½-in. water seal was used on the chloropicrin treatments. One series of 6 crocks was left untreated to serve as controls. Three weeks after treatment, 3 rose cuttings (*Rosa* sp. var. Dr. Huey) were planted in each crock, and the crocks were randomized on a greenhouse bench.

The plants were thinned to 2 per crock after 1 month because some in each lot failed to grow. The first nematode counts were made 4 months after treatment, at which time the foliage was cut back to 3 buds per plant and its dry weight (180°F for 12 hours) recorded. Soil for nematode counts was obtained by means of a soil tube ¾ in. in diameter. Two 50-cc soil samples from each replication were placed on a modified Baermann funnel (5) for 3 days. Similar nematode counts and top weights were then taken at approximately 6-week intervals for the duration of the experiment. The experiment was terminated at the end of 1 year. Observations were made on the root systems, nematodes were extracted from the roots (two 1-g samples from each replication on a modified Baermann funnel for 7 days), and dry weights were taken of the entire plant. Roots from each series were also cultured for bacteria.

Results.—Plants in the treated-soil series made better growth than did those in the control series throughout the experiment, and there was a very marked difference between the plants in treated soil and those in the untreated soil at the end of the experiment (Fig. 1). Chlorosis of the leaves began to appear in the control plants just before the first cutting; it became more severe as the experiment progressed, and at the termination of the experiment many plants in the untreated soil appeared to be dying. The chlorosis was interveinal; a very light green area was surrounded by the darker, normal green around the veins. All of the plants in the controls exhibited this chlorotic, stunted condition.

The root systems of the plants in the treated soil

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[2] Observed by the author in 1955.

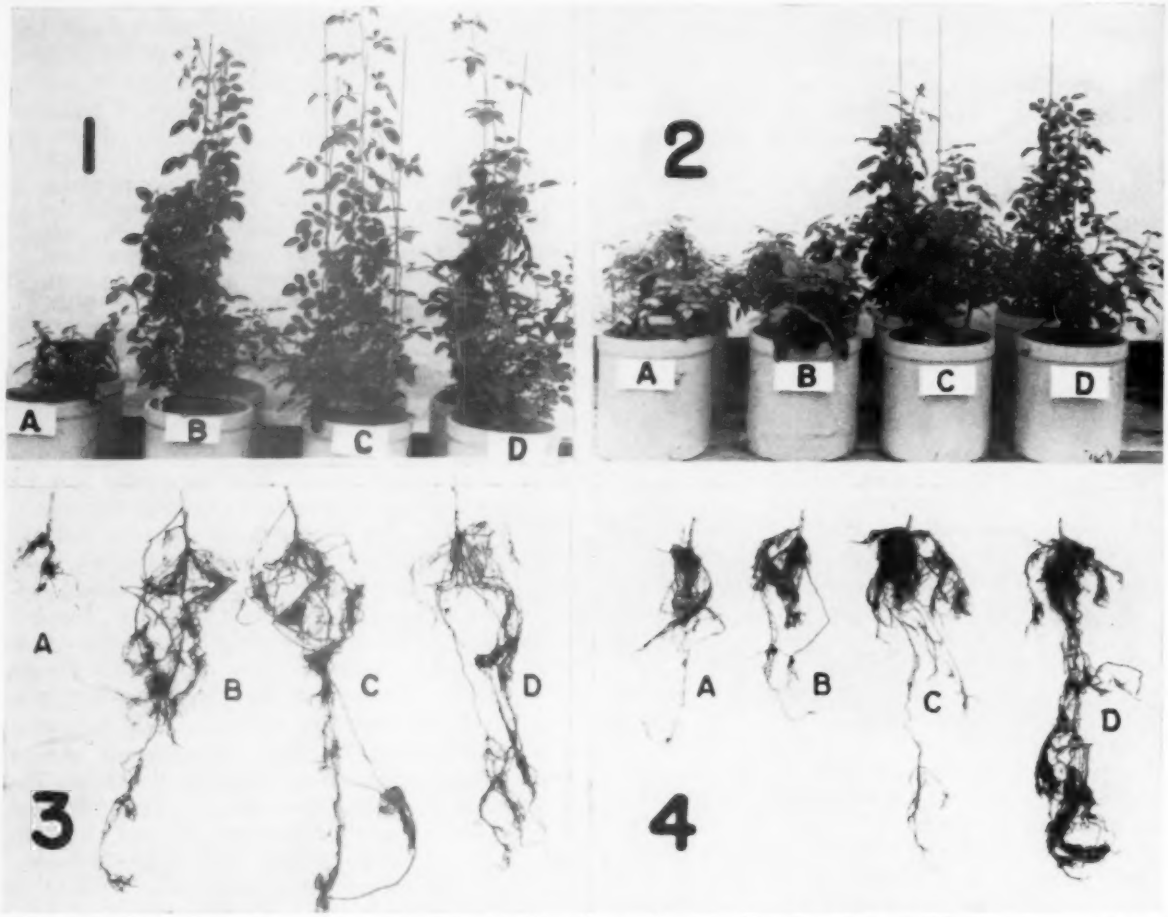

FIG. 1–4. Rose plants (tops and roots) at termination of experiments.—FIG. 1 and 3. Soil-fumigation experiment: A) nonfumigated control; B) D-D fumigated; C) Dowfume W-85 fumigated; D) chloropicrin fumigated.—FIG. 2 and 4. Soil-infestation experiment: A) *Pratylenchus vulnus* alone; B) *P. vulnus* and *Trichodorus christiei*: C) *T. christiei* alone; D) noninfested control.

were similar except for those of the Dowfume W-85 treatment, which exhibited crown gall and/or hairy root symptoms. There were no visible differences in size of the root systems of the plants treated with the 3 different soil fumigants (Fig. 3, B-D). Root systems of all plants grown in the treated soil had many clean-appearing feeder roots. Plants grown in untreated field soil had small root systems that were dark and necrotic in appearance and devoid of feeder roots (Fig. 3, A). The differences between the total dry weight of the untreated controls and that of plants in each of the 3 fumigated series were statistically significant at the 1 per cent level (Table 1).

Bacteria could be isolated only from the control plants and from plants grown in soil treated with Dowfume W-85. The species isolated appeared to be culturally similar to *Agrobacterium tumefaciens* (E. F. Sm. & Towns.) Conn and *A. rhizogenes* (Riker et al) Conn.

The population of *Pratylenchus vulnus* gradually increased in the soil in the untreated crocks and was at its highest level at the termination of the experiment (Table 1). *Trichodorus christiei* maintained itself for about 9 months in these crocks but was not recovered from the soil after a year (Table 1).

SOIL-INFESTATION EXPERIMENT.—*Materials and methods.*—Three rose cuttings (var. Dr. Huey) were planted in steam-sterilized loam in each of 16 3-gal. glazed crocks. Three months after the cuttings were planted, the crocks were divided into 4 series. Four hundred specimens of *Pratylenchus vulnus* were added to the soil of the first series, 400 of *P. vulnus* plus 400 of *Trichodorus christiei* to that of the second series, 400 of *T. christiei* to the third series, and 50 ml of distilled water to the fourth series. The nematodes in 50 ml of distilled water were poured around the base of each plant. Individual nematodes were picked out under a dissecting microscope by means of a bamboo splinter and put into distilled water. The nematodes were obtained by washing rose-field soil obtained from Ontario, California, through screens and placing the washings on a modified Baermann funnel. The crocks were randomized on a greenhouse bench.

Four months after infestation, nematode counts were made, the foliage was cut back to 3 buds, and dry weight of foliage was recorded as in the previous

TABLE 1.—*Growth of rose plants in the greenhouse in 3-gal. crocks containing soil infested with nematodes (Pratylenchus vulnus and Trichodorus christiei) and in soil fumigated with various fumigants*

Treatment	First count and cutting			Second count and cutting			Third count and cutting			Termination of experiment			
	Nematodes/100 cc of soil		Dry weight of plant tops (g)	Nematodes/100 cc of soil		Dry weight of plant tops (g)	Nematodes/100 cc of soil		Dry weight of plant tops (g)	Nematodes/100 cc of soil		*P. vulnus* in 1 g of roots	Total dry plant weight (g)[a]
	P. vulnus	*T. christiei*		*P. vulnus*	*T. christiei*		*P. vulnus*	*T. christiei*		*P. vulnus*	*T. christiei*		
						Soil-fumigation experiment							
Chloropicrin	0	0	10.8	0	0	25.4	0	0	29.5	0	0	0	150.7**
D-D	0	0	6.2	0	0	24.9	0	0	26.5	0	0	0	131.1**
Dowfume W-85	0	0	4.7	0	0	26.9	0	0	24.9	0	0	0	122.5**
None	7.7	0.3	0.6	302	0.3	4.9	183	1	16.4	841	0	67	53.7
						Soil-infestation experiment[b]							
P. vulnus	4,201	0	27.2	626	0	13.2	980	0	23.2	38	0	168	67.2**
P. vulnus + *T. christiei*	3,177	0	25.4	870	6	11.1	262	0	23.7	96	0	98	64.0**
T. christiei	0	28	25.9	0	11	22.0	0	0.5	35.7	0	0	0	101.9
None	0	0	28.3	0	0	24.4	0	0	41.1	0	0	0	106.5

[a] Double asterisks indicate statistical significance at the 1% level.
[b] Nematodes added to steam-sterilized soil.

test. Nematode counts and top weights of plants were taken at approximately 6-week intervals for the duration of the experiment. The test was terminated 1 year after the addition of the nematodes; observations were made on the root systems, and dry weights were taken of the entire plants. Rose leaves of the 4 series were analyzed at the termination of the experiment for minor elements and minerals.

Results.—No difference was noted in the appearance of the tops of the plants until after the first cutting. There was very little difference in the dry weight of all the lots in the first cutting (Table 1). After the second cutting, chlorosis of the leaves and a stunting of some of the plants began to appear in the series infested with *Pratylenchus vulnus* and in that infested with *P. vulnus* plus *Trichodorus christiei*. Leaf chlorosis and stunting of the rose plants in the 2 lots infested with *P. vulnus* became more pronounced as the experiment progressed. Nine months after addition of the nematodes, the plants of the 2 Pratylenchus-nematode series were all severely chlorotic and stunted. Leaf chlorosis (Fig. 5) was similar to that of plants in untreated soil in the first experiment. The plants in soil infested with *T. christiei* were similar in appearance to the controls. At the end of the experiment, there was a marked difference between the plants in the 2 series infested with *P. vulnus* and the plants in the 2 series without *P. vulnus* (Fig. 2).

The root systems of the plants in soil infested with *Pratylenchus vulnus* alone were necrotic, with no evidence of new growth, and feeder roots were sparse at the end of a year (Fig. 4, A). The plants in soil infested with both *P. vulnus* and *T. christiei* were similar in appearance to those in soil to which *P. vulnus* was added (Fig. 4, B). The plants in soil infested with *T. christiei* alone had more extensive root systems than did those of the 2 preceding series, and light-colored feeder roots were numerous (Fig. 4, C). Root systems of plants grown in noninfested soil were similar to those of plants grown in soil containing *T. christiei* (Fig. 4, D). The total dry weight of plants of the 2 series to which *P. vulnus* was added was significantly less than that of the controls (Table 1). There was no significant difference between the total dry weight of plants grown in soil containing *T. christiei* and that of the controls (Table 1).

The population of *P. vulnus* increased very rapidly in the 2 series where this species was added to the soil. There was a gradual decline in the population after the high level reached at 5 months. The population of *T. christiei* maintained itself for 9 months but was not recovered at the termination of the experiment (Table 1).

Leaves in the 4 series showed a division into 2 groups on the basis of leaf analysis. Leaves of plants in the Pratylenchus-nematode series were deficient in iron, copper, and potassium and were higher than those of the other 2 series in zinc, calcium, magnesium, and nitrogen. Leaf content of phosphorus, sodium, manganese, and molybdenum did not differ in the 4 series (Table 2).

TABLE 2.—*Mineral constituents of composite leaf samples from rose plants grown in nematode-infested and in noninfested soil in 3-gal. crocks in the greenhouse*

Inoculum	Percentage of dry weight						ppm				
	Mg	Na	Ca	K	P	N	Zn	Cu	Mn	Fe	Mo
Pratylenchus vulnus	0.497	0.112	5.68	1.060	0.553	3.89	51.0	3.5	404	46.8	0.21
P. vulnus + *Trichodorus christiei*	0.432	0.93	5.15	1.054	0.639	3.45	34.0	3.4	444	62.0	0.26
T. christiei	0.405	0.099	4.23	1.336	0.556	3.22	27.5	4.9	468	85.8	0.32
None	0.406	0.119	4.52	1.418	0.530	3.28	24.0	4.5	434	81.0	0.38

DISCUSSION.—Results of the first test indicate that the disease of roses can be eliminated by soil fumigation before planting. Two of the 3 soil fumigants used are good nematocides but poor fungicides. The elimination of the disease by these chemicals indicates that nematodes may be the cause of this disease. The recovery of bacteria in the Dowfume W-85 treatments from plants with no top symptoms or with normal-sized root systems indicates that these bacteria do not cause this disease. Bacteria were not recovered from plants of the D-D or chloropicrin treatments, and plant weights and lack of disease symptoms were similar in all 3 fumigation treatments. Only the plants in soil infested with *P. vulnus* exhibited the chlorotic and stunting effect of this disease.

In the second test, the disease was produced only by infestation of soil with *Pratylenchus vulnus* and of *P. vulnus* plus *Trichodorus christiei*. The probability that another agent responsible for this disease was introduced along with *P. vulnus* is small, since the addition of *T. christiei* did not produce any diseased plants. It has been reported for 1 nematode that the internal contents are sterile (4). Contaminants that might be introduced would very likely be on the body surface. *P. vulnus* and *T. christiei* from the same soil would probably have the same body-surface contaminants.

There was more root destruction in the untreated soil than where *P. vulnus* was added to steam-sterilized soil. This might be expected because of the activity of secondary pathogens present in field soil. Roots in the infested series also had a 3-month period of growth in steam-sterilized soil before *P. vulnus* was introduced, whereas in the first test the cuttings were placed directly into infested field soil.

The visible symptoms seen in plants were documented by leaf analyses that showed marked differences in iron, copper, zinc, potassium, nitrogen, and calcium between Pratylenchus-inoculated plants and Pratylenchus-free plants.

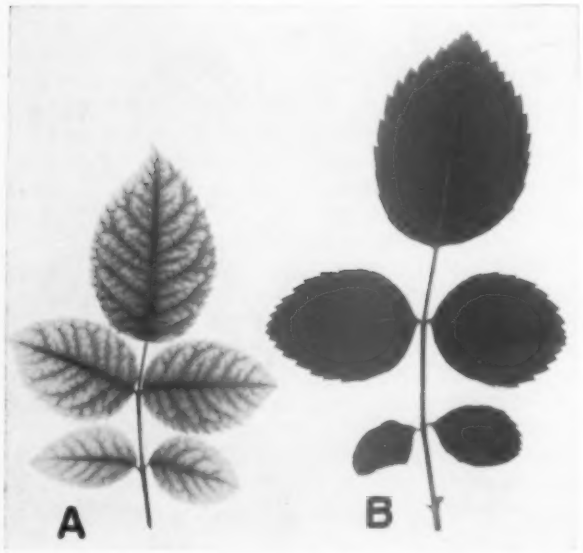

FIG. 5. Leaves from rose plants of soil-infestation experiment: A) chlorotic leaf from plant grown in soil infested with *Pratylenchus vulnus;* B) healthy leaf from plant grown in noninfested soil.

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A HISTOLOGICAL STUDY OF THREE HELMINTHOSPORIUM LEAF BLIGHTS OF CORN [1]

Peter R. Jennings [2] and Arnold J. Ullstrup [3]

SUMMARY

Spore germination and penetration by *Helminthosporium turcicum*, *H. maydis*, and Race I of *H. carbonum* were similar on leaves of susceptible and resistant corn plants. Eighty to 90 per cent of all penetrations were direct, with the majority occurring 6–18 hours following inoculation.

H. turcicum became established early in susceptible xylem tissue, with mycelial growth progressively filling vessels and tracheids. Distinct wilt symptoms and chlorenchyma necrosis followed extensive xylem plugging, and hyphae moved out of xylem into moribund parenchyma only after lesions formed. Observations indicated that this blight is fundamentally a localized wilt disease; the absence of pectin plugs in diseased material suggested that plugging of the water-conducting elements by the fungus was responsible for the wilting. The similarity of symptoms and histology of other hosts of *H. turcicum* to those of corn indicated that pathogenesis in those plants was the same as in corn.

Resistance to *H. turcicum* apparently was located in xylem tissue and was not a function of host structure. Its effect was to inhibit mycelial growth so that wilting and necrosis were delayed, resulting in fewer and smaller lesions than occurred in susceptible tissue.

H. maydis and *H. carbonum* ramified rapidly through susceptible parenchyma, but vascular tissues were not invaded. Host necrosis was closely associated with the invading mycelium. Lateral growth of *H. maydis* was eventually blocked by large vascular bundles that conferred the characteristic parallel-sided appearance to lesions.

Growth of *H. maydis* in resistant chlorenchyma was effectively impeded following penetration. Hyphae, which was confined about the penetration point, elicited only localized host reactions. Penetration pegs of *H. carbonum* were typically held within the penetrated epidermal cells of resistant leaves, with little or no growth extending into the subtending chlorenchyma.

Three leaf diseases of corn (*Zea mays* L.) incited by species of *Helminthosporium* occur throughout most of the eastern half of the United States as well as in other corn-producing areas of the world. Northern corn leaf blight incited by *H. turcicum* Pass. and southern corn leaf blight incited by *Cochliobolus heterostrophus* Drechsl. (*H. maydis* Nisikado & Miyake) are of appreciable economic importance. Race I of *H. carbonum* Ullstrup, which incites Helminthosporium leaf spot, is the most virulent of these pathogens, but it is of minor practical consequence because only a few inbred lines are susceptible and resistance is governed by a single dominant gene. The 3 diseases have been studied from the standpoints of inheritance of resistance (5, 6, 8, 13) and their effects on grain yield (14). Wide differences in resistance to the diseases are apparent among inbred lines in the young- and adult-plant stages, but only in the case of Helminthosporium leaf spot are such differences clearly defined in the young-seedling stage. There appears to be little correlation of resistance to the 3 diseases among inbred lines.

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The objectives of the present study of the 3 diseases were to compare the host-parasite relations and to determine any histological differences between resistant and susceptible inbred lines that might account for their disease reactions.

MATERIALS AND METHODS.—Early stages of infection were observed *in toto* on young and mature leaves that had been inoculated by spraying with spore suspensions. Pieces of leaves were cleared in a mixture of absolute alcohol and glacial acetic acid and stained with analine blue in lactophenol. Similar material for serial sections was fixed in Karpechenko's solution, dehydrated in glycerol followed by 95 per cent ethanol and 1-butanol and embedded in paraffin. Sections cut about 12 μ in thickness were stained in safranin and fast green. Mesocotyls of seedlings inoculated with a concentrated spore suspension of *H. carbonum* were fixed, embedded, and stained in the same manner.

The inbred lines examined for reactions to *H. turcicum* were C123 and L97 (resistant) and Hy, K64, and 1456-6[4] selfed (susceptible). Those examined for reactions to *H. maydis* were C103 and MoG (resistant) and Wf9 and Prl (susceptible). The single crosses used for *H. carbonum* were K61 × Prl (resistant) and K61 × Pr (susceptible).

RESULTS.—*Northern corn leaf blight.*—Spore germination and penetration by *H. turcicum* were similar on resistant and susceptible leaves of both young and mature plants. Maximum germination and penetration occurred 12–18 hours after inoculation. About 90 per

[4] 1456-6 was an extremely susceptible segregate from an F_2 population of Oh28 × Oh4c.

cent of all penetrations were direct and typically occurred over the juncture of vertical epidermal walls, whereas 10 per cent were stomatal. Elliptical appressoria, averaging 4.0 × 6.0 μ, were associated with all penetrations.

1) *Histology of infection of susceptible inbred lines.*—The first fungus cells to form in the penetrated epidermis or substomatal cavity were typically barrel-shaped and large, ranging up to 12 × 22 μ. Simple primary hyphae developed from these cells and extended into the chlorenchyma (Fig. 1, A). Limited intracellular growth continued in the chlorenchyma until the hyphae entered the sheaths and then the xylem of vascular bundles (Fig. 1, B, C), but further spread in chlorenchyma ceased once the bundles were penetrated.

Longitudinal growth through xylem tracheids and vessels was rapid in contrast to the initial dilatory progress of the parasite in the chlorenchyma. Hyphae were typically filiform, repeatedly branched, and large, ranging up to 12 μ in diameter (Fig. 1, D, F). Sclerenchyma and xylem parenchyma were occasionally parasitized, but phloem was not. The growth of hyphae from 1 bundle to another was accomplished through cross veins (Fig. 1, E). By approximately 14 days following inoculation, the xylem of the smaller bundles appeared to be totally blocked whereas a maximum of 80–90 per cent of the xylem area of the larger bundles was plugged with mycelium (Fig. 1, G, H).

Penetration typically elicited localized host responses, resulting in macroscopically visible flecks (Fig. 1, B). A breakdown of chlorenchyma chloroplasts and of nuclei and limited wall collapse occurred about most primary hyphae, but occasionally infection was effected without apparent host responses (Fig. 1, A). Growth in xylem elements resulted in no apparent tissue reactions until about 14 days following inoculation, when wilted areas appeared concurrent with extensive vascular plugging. The wilted areas enlarged rapidly and turned necrotic. Complete breakdown of chlorenchyma cells between parasitized bundles was associated with the onset of wilting. The growth of hyphae within the xylem continued in advance of the wilted areas until the lesions reached their maximum size. Hyphae grew out of the xylem into the necrotic chlorenchyma only after leaf tissue wilted (Fig. 1, I). Mycelium quickly filled the intercellular spaces and conidiophores emerged through stomata if adequate moisture was present.

The effect of infection on the transport of water was studied by the use of a 0.5 per cent aqueous solution of eosin dye. Dye conduction was followed in detached noninoculated leaves and in infected leaves with wilted, incipient lesions; this transport was verified by microscopic examination of free-hand sections. The dye was carried through veins of healthy leaves within 30 minutes. The flow was markedly to completely impeded in wilted tissue but was normal on either side of and distal to the wilted areas. The results suggested that the plugging of xylem critically inhibited the water flow through parasitized tissue.

The possibility that vascular plugs other than mycelium contributed to the impeded water flow was investigated by use of a stain specific for pectic materials. Serial and free-hand sections of flecked, wilted, and necrotic leaf tissue were stained for 2–3 hours in a 1:5000 aqueous solution of ruthenium red. No characteristic pectinaceous plugs were observed, suggesting that mycelium alone was responsible for the blocking of water transport.

2) *Histology of infection of resistant inbred lines.*—The histological pathology of resistant inbred lines from the time of fungus penetration to entrance of primary hyphae into vascular bundles was like that described for susceptible material (Fig. 1, A, B, C). Hyphae started to grow following xylem penetration of the resistant line, but both longitudinal and lateral growth was soon retarded. Also, the hyphae rarely branched and were smaller, averaging 3–4 μ in diameter (Fig. 2, A, B). Plugging of a limited number of smaller bundles, sufficient to cause localized blocking of water movement, occurred about 3–4 weeks following inoculation. The time required for some vessels to be plugged and the extent to which plugging took place depended upon the age of the resistant host. Young plants showed only moderate resistance, whereas mature leaves effectively inhibited fungus growth.

Host reactions attending infection of resistant plants were similar to responses observed in susceptible leaves; however, flecks persisted longer and the onset of wilting occurred much later. Hyphae grew out from the xylem following necrosis, and conidiophores emerged from stomata under favorable conditions.

3) *Histology of infection of other hosts of H. turcicum.*—Since *H. turcicum* incites a leaf blight of other host species, with symptoms similar to that observed on corn, the pathological histology of some of these hosts was examined from hand-sectioned naturally infected leaves. The Greenleaf, Oklahoma 8, Lahoma, Sweet 372, and Piper varieties of sudan grass (*Sorghum vulgare* var. *sudanense* Hitchc.), a sorghum selection from Standard White Durra (*S. vulgare* Pers.), and broomcorn (*S. vulgare* var. *technium* Jav.) were examined. Observations suggested that the pathological histology of *H. turcicum* on these hosts was the same as described for corn. Fungus growth was profuse in but limited to the xylem, with marked plugging of water-conducting elements by mycelium preceding wilting and necrosis.

Southern corn leaf blight.—Spore germination and penetration by *H. maydis* were similar on resistant and susceptible leaves of both young and mature plants. Maximum germination and penetration occurred within 18 hours following inoculation. From 80–85 per cent of all penetrations were direct, the remainder being stomatal. Elliptical appressoria, averaging 5.0 × 7.5 μ, were associated with all penetrations.

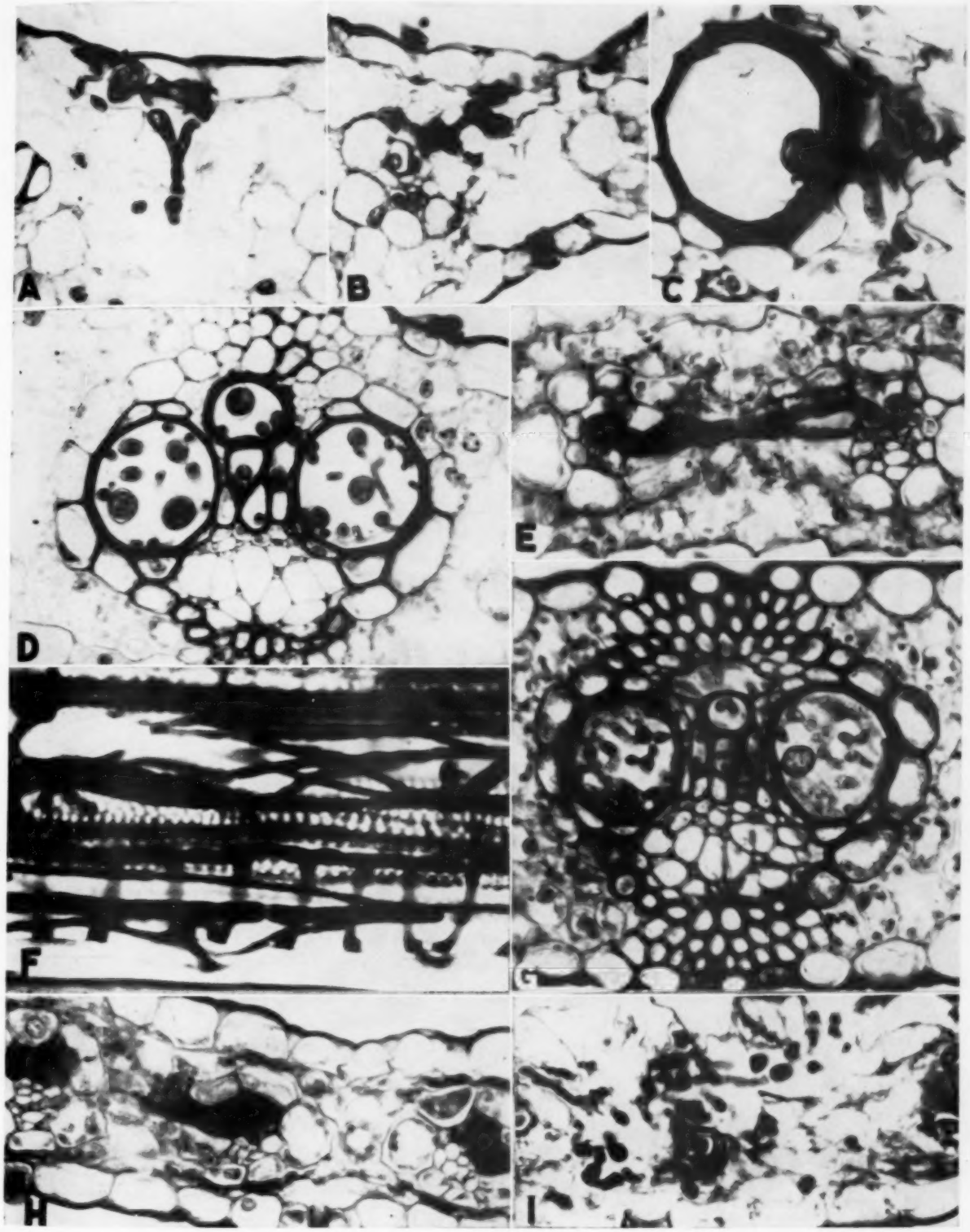

FIG. 1. *Helminthosporium turcicum*. A) Penetration hypha in chlorenchyma. B,C) Hyphae that were penetrating xylem tracheid and vessel. D,F) Growth in susceptible xylem 7 and 9 days after inoculation. E) Lateral spread through susceptible cross vein. G,H) Plugging of larger and smaller susceptible bundles. I) Emergence of hyphae from susceptible bundles into moribund parenchyma. (C, ×900; others, ×450.)

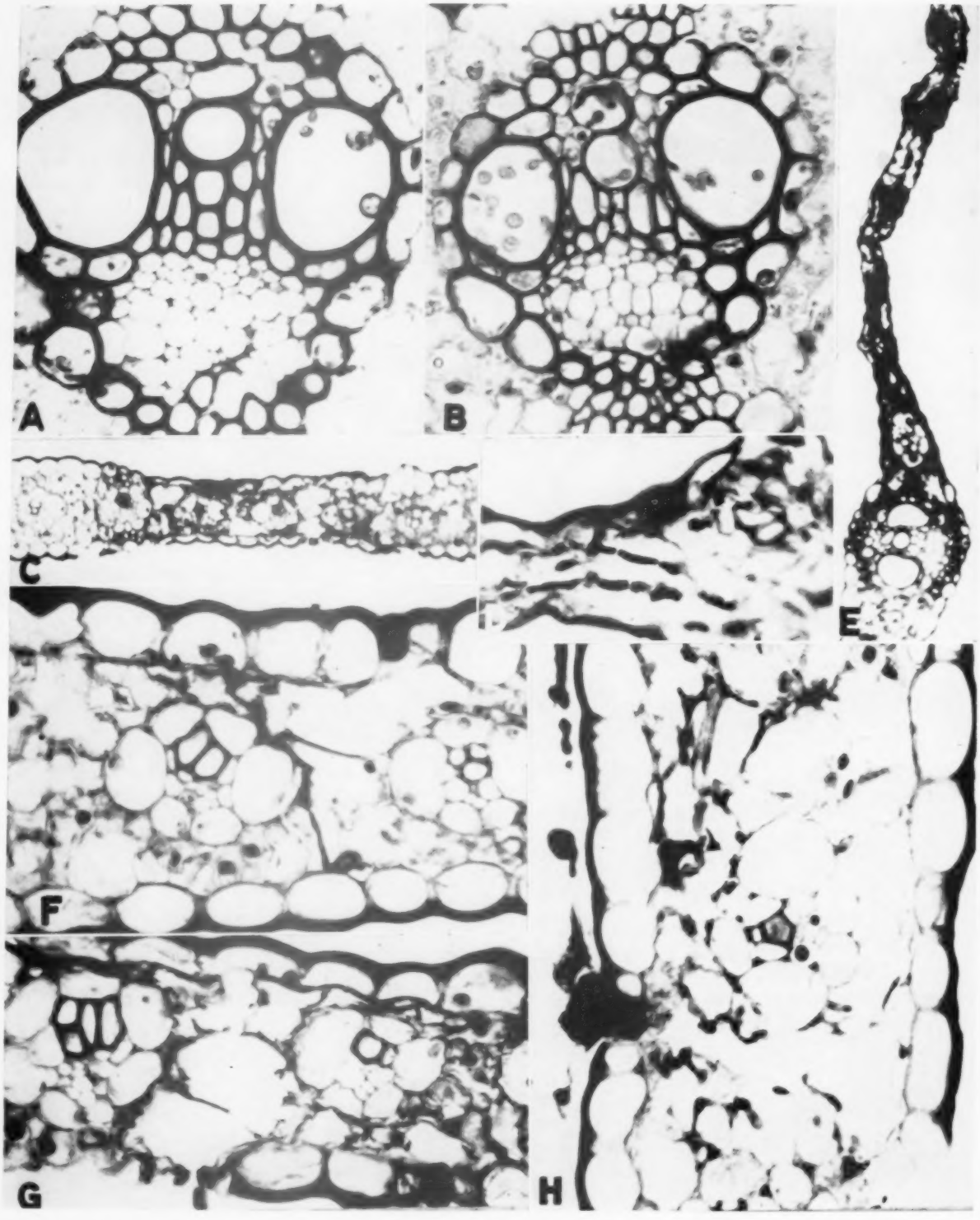

FIG. 2. A,B) *Helminthosporium turcicum*. C–H) *H. maydis*. A,B) Growth in resistant xylem 9 and 17 days after inoculation. C) Extent of host reaction of resistant leaf (compare with E). D) Growth in susceptible tissue following lesion formation. E) Limitation of lesion by large vascular bundles of susceptible leaf. F) Sparse growth following penetration of resistant leaf (compare with H). G) Resistant host reaction and confined fungus growth 8 days after inoculation. H) Ramification through susceptible chlorenchyma following penetration. (C and E, ×150; others, ×450.)

1) *Histology of infection of susceptible inbred lines.*—Infection hyphae branched and proliferated in the penetrated epidermal cells and substomatal cavities before entering chlorenchyma. Filiform hyphae, averaging about 2 μ, radiated into chlorenchyma from the penetration sites (Fig. 2, H), with host reactions closely following fungus progression. Chloroplast breakdown preceded cell-wall collapse. Hyphae within the expanding lesions ramified throughout the necrotic masses of cellular fragments, but vascular tissues were not parasitized (Fig. 2, D). Hyphae at the edges of lesions continued unimpeded longitudinal advance in chlorenchyma. Lateral growth, however, continued only until hyphae contacted the larger vascular bundles, which effectively blocked further extension (Fig. 2, E). Hyphae did not penetrate the xylem or phloem but occasionally entered the sclerenchyma or bundle sheaths. Thus, the limitation of parasitism and necrosis was due to the large leaf bundles that conferred the characteristic parallel-sided appearance on lesions.

2) *Histology of infection of resistant inbred lines.*—Infection hyphae branched once or twice but did not fill the penetrated epidermal cells or sub-stomatal cavities, and only 1 or 2 hyphae grew out from each infection site into the chlorenchyma of resistant inbred lines (Fig. 2, F). The hyphae averaged about 2 μ in diameter and consisted of short filaments rather than long mycelial strands. The fungus was held within a few chlorenchyma cells about the penetration site (Fig. 2, G). Vascular tissues were not parasitized.

Host reactions to infection were similar to those found in susceptible leaves although not nearly so extensive. Cell collapse and necrosis were confined closely about the sites of penetration (Fig. 2, C, G). Macroscopically, these resulted in enlarged chlorotic flecks with necrotic centers. Little or no growth occurred in necrotic resistant tissue in contrast to the abundant growth within necrotic cellular debris in lesions on susceptible leaves. No morphological barriers that could account for, or contribute to, resistance were observed. Observations *in vivo* and of fixed tissues indicated that young plants were only moderately resistant following inoculation. Inhibition of fungus development increased with age, so that fully mature plants possessed marked resistance.

Helminthosporium leaf spot.—Spore germination and penetration by *H. carbonum* were similar on resistant and susceptible leaves. Most penetrations occurred about 12 hours following inoculation. About 90 per cent of all penetrations were direct, the remainder being stomatal. All were effected by means of circular to clavate appressoria that ranged up to 5–14 μ in diameter.

1) *Histology of infected leaves of a susceptible single cross.*—Infection hyphae branched freely and filled the penetrated epidermal cells before septate primary hyphae grew intracellularly into the subtending chlorenchyma. Mycelial branches from the primary hyphae averaged about 4 μ in diameter and ramified through the chlorenchyma (Fig. 3, A). Nuclei and chloroplast breakdown and cell-wall collapse, like that observed in susceptible host tissue invaded by *H. maydis*, closely followed mycelial spread. Hyphae proliferated within the necrotic tissues (Fig. 3, G) and continued to advance at the edges of the expanding lesions. Lateral growth was temporarily blocked by the large vascular bundles, but hyphae were able eventually to pass through the sclerenchyma bordering the bundles. Parasitism of the phloem was never observed, and penetration of xylem tracheids and vessels occurred rarely.

2) *Histology of infected leaves of a resistant single cross.*—Hyphae established in epidermal cells following direct penetration often branched 1 or more times but rarely filled the cells with mycelium. The hyphae averaged about 4 μ in diameter and appeared like those within susceptible epidermal cells. With few exceptions, however, all growth was confined to the initially penetrated epidermal cells (Fig. 3, C, D). Very rarely a hyphal peg extended intracellularly for 20–30 μ into subtending chlorenchyma (Fig. 3, E).

Stomatal penetration was followed by an extension of the penetration peg into the substomatal cavity for 10–25 μ, but growth was quickly inhibited. Observations suggested that the inhibited hyphae within epidermal cells were not killed. To verify this, chlorotic flecks were dissected from resistant leaves and plated on potato-dextrose agar at several intervals following inoculation. The fungus was consistently recovered from such flecks for a minimum of 60 days after inoculation, indicating that the inhibited mycelium remained viable.

Careful observations of *in toto* mounts and microtome sections indicated that host reactions developed no sooner in the resistant than in susceptible leaves. Epidermal walls of cells about the infection site often became conspicuously thickened (Fig. 3, D). Chlorenchyma chloroplasts below the inhibited hyphae disintegrated, whereas protoplasm appeared distinctly granular and assumed an abnormal reddish color when stained with safranin (Fig. 3, E). A few chlorenchyma cell walls occasionally collapsed, resulting in the visible chlorotic flecks.

3) *Histology of infected mesocotyls of a susceptible single cross.*—Direct penetrations typically occurred over the vertical walls of adjacent epidermal cells. Established hyphae soon initiated rapid intracellular growth so that the outermost cortical cells were often completely filled with mycelium averaging about 3.5 μ in diameter (Fig. 3, B). Centripetal spread continued until the endodermis was reached. This layer, however, impeded growth to such an extent that the pericycle, xylem, and phloem were rarely penetrated. In the later stages of parasitism, large macrohyphae, averaging about 7.5 μ in diameter, developed in the epidermal and outer cortical layers. Conidiophores emerged through the epidermis from these macrohyphae.

Initial penetration was usually accompanied by a

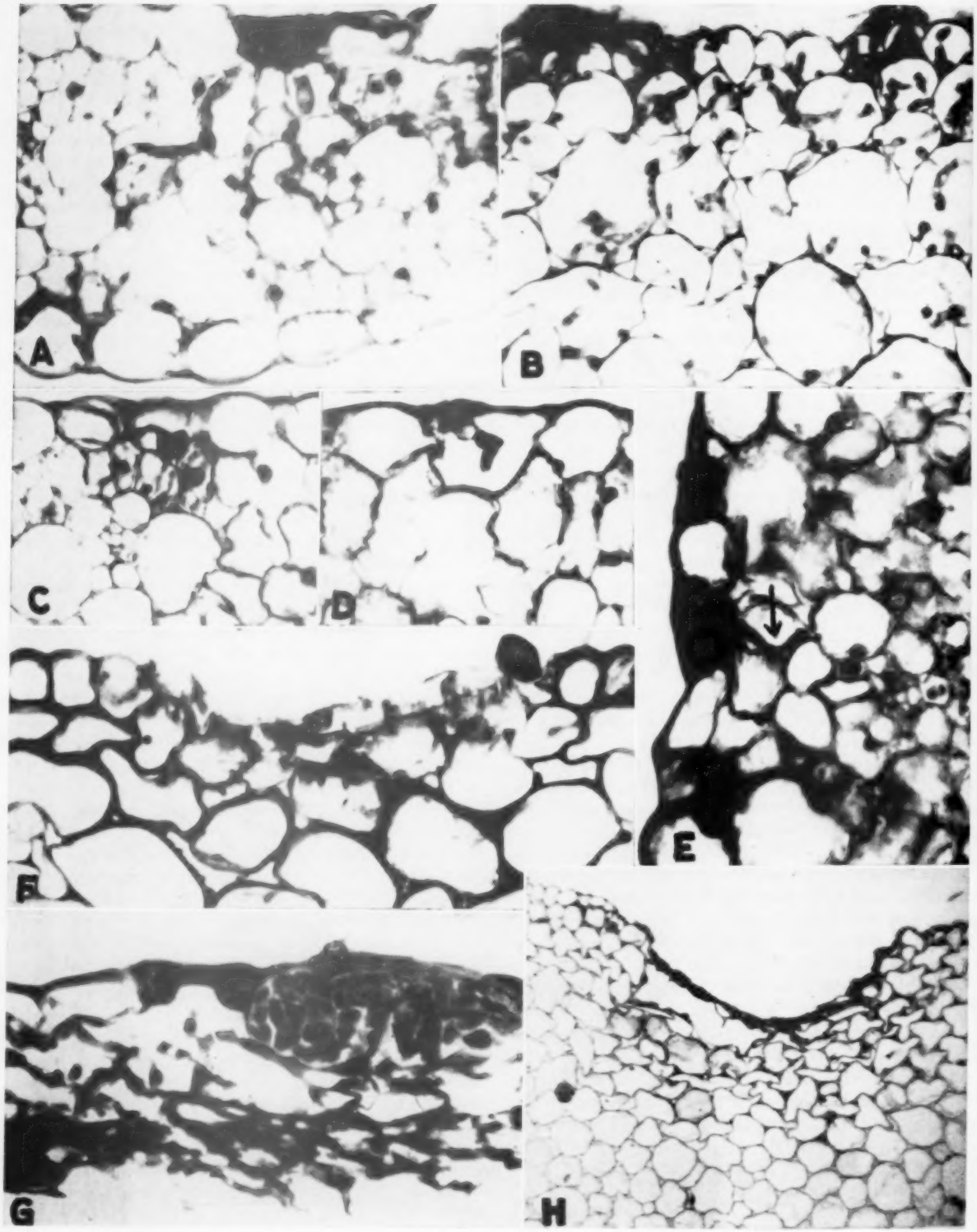

FIG. 3. *Helminthosporium carbonum*. A) Growth following penetration of susceptible leaf. B) Growth in susceptible mesocotyl. C,D) Inhibited penetration hyphae in epidermal cells of resistant leaf. E) Growth of hyphal peg into chlorenchyma of resistant leaf. F) Early stage of pit formation in resistant mesocotyl. G) Heavy parasitism of necrotic susceptible leaf tissue. H) Final pit in resistant mesocotyl. (H, ×150; others, ×450.)

slight thickening of the epidermal walls and a faint cytoplasmic staining reaction about the penetration peg. Lignituber formation in cell walls often attended penetration. Spread in the cortex caused little visible host response. Nuclei and cytoplasm about hyphae disintegrated, but cell walls remained unaffected.

4) *Histology of infected mesocotyls of a resistant single cross.*—In the resistant host, penetration was often accompanied by the formation of lignitubers and was followed by limited growth in the outer cortex. Within 48 hours, a rapid cellular degeneration with concomitant pit formation took place. Initially, several epidermal and cortical cells about the sparse fungus growth showed abnormally thickened cell walls and partial compression (Fig. 3, F), whereas cytoplasm and nuclei stained deeply with safranin before disintegrating. Finally, cellular collapse resulted in a pit with a depth of about 7 or 8 cortical cells (Fig. 3, H). Hyphae were completely inhibited about the pits following the initial penetration of a few cortical cells.

DISCUSSION.—The pathological histology of many leaf diseases caused by species of *Helminthosporium* and other fungi has been investigated. In these, the growth of the parasites is confined chiefly to chlorenchymatous tissue, with occasional penetration of vascular elements. This is characteristic of *H. victoriae* Meehan & Murphy on oats (*Avena sativa* L.) (7) and of *H. leucostylum* Drechsl. on *Eleusine indica* Gaertn. (12), which grow well in chlorenchyma and the phloem but not in the xylem. *H. gramineum* Rab. grows in the xylem of mature barley (*Hordeum vulgare* L.) leaves, but chlorenchyma is the major site of invasion (11). The parasitism of both *H. maydis* and *H. carbonum* on corn leaves is confined almost entirely to chlorenchyma. *H. turcicum*, however, is unusual in that growth of parasitic mycelium is most profuse in xylem tissue. Symptomatology, eosin dye studies, and histological observations indicated that this blight is basically a localized wilt disease. A report by Cox and Wolf (2) of an unusual crown rot of sweet corn supports this conclusion. They noticed sudden wilting that culminated in plant death in 2–5 days, and examination of the crown showed that discoloration was initially restricted to vascular bundles.

The pathogenesis of wilt diseases has recently received attention. Waggoner and Diamond (15) concluded that the reduction in water flow by mycelium of *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. & Hans. in wilt-affected tomato (*Lycopersicon esculentum* Mill.) stems probably is less than 5 per cent. Gothoskar et al (4) found that pectin methyl esterase and a depolymerase produced typical wilting and browning associated with Fusarium wilt of tomato. They concluded that wilting resulted from plugging of vessels by degradation products of the cell wall that were liberated by the action of pectic enzymes secreted by the fungus. Pierson et al (9) observed that the vascular plugs stained with ruthenium red, an indication that they were composed at least in part of pectic materials. Recent work (10, 1) indicates that the mechanism of wilting in Verticillium wilt of tomato and in the Dutch elm disease may be similar to that of Fusarium wilt of tomato.

The absence of pectin plugs in host tissues stained with ruthenium red suggested that pectic enzymes probably are not involved in lesion formation on corn leaves parasitized by *H. turcicum*. The extensive vascular plugging by hyphae indicated that mycelium alone was responsible for wilting by physically obstructing water flow. Similarity of symptoms and histology of other susceptible hosts indicated that pathogenesis in these species may be the same as in corn.

Resistance to *H. turcicum* was not evident prior to the establishment of hyphae in the xylem. The resistant principle, apparently in the xylem, did not appear to be a function of host structure. Resistance became progressively more active as the host plant increased in age.

H. maydis and *H. carbonum*, unlike *H. turcicum*, apparently do not incite localized wilting, since vascular plugging was not observed. Both fungi ramified exclusively in chlorenchyma, but lateral spread of *H. maydis* was effectively checked by large vascular bundles. The growth of *H. maydis* was arrested soon after penetration of resistant chlorenchyma, but the degree of inhibition was not so pronounced as that observed where *H. carbonum* penetrated resistant host tissue, since penetration pegs of the latter fungus were held within epidermal cells. This high degree of resistance, which is inherited as a monogenic dominant, suggests the advisability of further studies on the nature of resistance. No differences were noted in the rapidity with which resistant and susceptible leaves reacted to penetration by *H. carbonum*. This parallels recent observations (3) that rate of host reactions is not a dependable indicator of susceptibility or resistance of potatoes to late blight.

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CARNATION VIRUSES IN THE UNITED STATES[1]

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SUMMARY

The term "yellows" for double infections of carnations with mosaic and streak viruses is considered misleading inasmuch as symptoms caused by these 2 viruses are merely additive and are not suggestive of a yellows disease. Confusion of streak with feeding injury by *Myzus polaris* Hille Ris Lambers is suggested as an explanation of the unconfirmed report of aphid transmission of carnation streak virus. Carnation streak virus was transmitted by grafting but not by *Macrosteles fascifrons*. Western aster yellows inoculated into carnation by dodder caused curvature of leaf tips initially, but the virus failed to persist in carnation. Carnation mosaic virus is separable from mottle and ringspot viruses by transmission by means of *Myzus persicae* to *Dianthus barbatus*. Carnation mottle virus at high dilution infects *Chenopodium amaranticolor*, with symptoms appearing in 6 to 7 days. Carnation ringspot virus is detected 4 or 5 days after juice inoculation to *Gomphrena globosa*; it infects many other plants. Prevalence (in percentage) of these viruses in 151 carnation varieties from 8 states was streak, 11; mosaic, 3; mottle, 92; and ringspot, 1. Only mottle virus was transmitted by root contact; only mottle and ringspot viruses were transmitted by the cutting knife; only mosaic virus was transmitted by *Myzus persicae*; no virus was transmitted by *M. polaris*.

American literature contains a number of conflicting statements about carnation (*Dianthus caryophyllus* L.) viruses. Köhler and Klinkowski (19) found this literature difficult to evaluate; in reporting on carnation viruses in England, Kassanis (17) felt it better to assign new names to his isolates rather than to try to relate these to American descriptions. During recent years, R. N. Stewart of the Ornamental Plants Section assembled a number of carnation varieties from 8 states for screening for resistance to bacterial and Fusarium wilts. These varieties furnished us an opportunity to estimate prevalence of viruses in American commercial carnations and to study means of transmission of the viruses present. The results of these studies, together with our interpretation of some of the conflicts in earlier literature, are reported.

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Review of literature.—Peltier (22) and Lamkey (20), in pioneer studies of carnation viruses in Illinois, graft-transmitted to carnation a virus or viruses evidently including the virus now known as streak virus. They applied the name "yellows" to the disease they studied, perhaps because success of graft-transfer and failure of sap-transfer of the causal agent suggested a resemblance to peach yellows, the best known disease of that class at that time. Creager (6) introduced the name carnation mosaic. Jones (14) recognized streak and mosaic as separate diseases, but retained the name "yellows" for double infections. This terminology was continued by subsequent writers (7, 10, 11, 23). In our experience the symptoms of mosaic and streak are merely additive in double infections, and the name "yellows" is therefore misleading rather than descriptive or useful.

Jones (14) reported transmission of the streak virus, but not the mosaic virus, by *Myzus persicae* (Sulz.). In our experiments (4), not published in detail, *M. persicae* proved an efficient vector of the

mosaic virus but failed to transmit the streak virus. We feel that Jones must have used *Myzus polaris* Hille Ris Lambers in his tests. This latter species causes direct feeding injury (Fig. 1) in carnation leaves in the form of yellowish spots that later turn brown and necrotic, but it fails to transmit any of the carnation viruses discussed herein. These aphids thus injure young leaves, but subsequent growth of the injured plants after removal of the aphids places the injured leaves near the base of the plant so that resemblance of aphid injury to streak becomes closer. Living *Myzus polaris* is recognizably different from living *M. persicae*, but cleared specimens of the 2 species mounted for taxonomic study are hard to distinguish. For this reason, most aphid specialists have regularly assigned *M. polaris* to *M. persicae*. The present writers (4) maintained that the carnation aphid is distinct from typical *M. persicae* and suggested that *Myzus dianthi* Schrank might be the correct designation. In September, 1956, Hille Ris Lambers examined preserved specimens collected by the writers from carnations in several states and concluded that these were the same as his *M. polaris* from Iceland. He declared that *M. dianthi* Schrank is a synonym of *M. persicae* Sulz.; however, the writers have been unable to establish colonies of typical *M. persicae* on carnation, the host from which *M. dianthi* was described. Although *M. persicae* does not ordinarily colonize on commercial varieties of carnation, after preliminary starvation it will pick up carnation mosaic virus by a few minutes' feeding on infected carnation and will transmit the virus to carnation or *Dianthus barbatus* L. in the nonpersistent manner. When the aphids are allowed to migrate naturally from the infected leaf pieces, the percentage transmission is high.

The carnation aphid, *M. polaris*, was formerly common on greenhouse carnations, causing spotting and dwarfing of plants and blasting of flower buds. It is restricted to carnation and closely related hosts as food plants. It severely injures the carnation varieties Arundel, Marchioness of Headfort, and Puritan, and injures others less severely. Following the use of organic phosphorus insecticides for spider mite control, the carnation aphid, also vulnerable to these materials, has become comparatively rare in Middle Atlantic and Mid-Western states. In areas with cooler summers, such as New England and the Pacific Northwest, this aphid is still seen, presumably because the practices of spider mite control are less rigorous in such areas.

Ames and coworkers (1, 2) reported properties and electron microscopy of carnation mosaic virus. Noordam et al (21) published similar studies on anjermosaiek virus, known to English (17) and American workers (5) as carnation ringspot virus. Thomas (27) noted strains of carnation mosaic virus differing in symptom production in *Dianthus barbatus*. Kassanis (15) described a latent virus of carnation detectable by serology and electron microscopy and in 1955 (17) presented data on properties, serology, and electron microscopy of 4 viruses from carnation, namely latent, ringspot, mottle, and vein mottle viruses.

Test plants recommended by various writers for detection of carnation viruses are *Chenopodium album* L. (9), *C. amaranticolor* Coste & Reyn. (12), *Dianthus barbatus* (4), *D. superbus* L. (10), and *Gomphrena globosa* L. (32). Additional hosts of carnation mosaic virus were reported by Gasiorkiewicz (8) and hosts of streak and mosaic viruses by Rumley and Thomas (23) and Thomas (25). Root transmission of carnation mosaic virus was reported by Baker and Thomas (3) and Thomas and Baker (28); transmission of this virus on the cutting knife was reported by Rumley and Thomas (23).

Thomas and Daniels (29) reported transmission of streak virus from carnation to China aster (*Callistephus chinensis* (L.) Nees) by grafting and also return transfer of the virus from China aster to carnation by the same method. These workers also reported transmission of aster yellows virus from China aster to carnation, which developed streak symptoms, and from carnation to China aster, by the six-spotted leafhopper, (*Macrosteles fascifrons* Stal.). Later, Thomas (26) attributed to aster yellows virus a flower malformation that appeared in Hercules carnation at day temperatures above 80°F. Smith and Brierley (24) failed to confirm this relation of streak to aster yellows virus or to transmit streak virus with this leafhopper.

EXPERIMENTAL RESULTS.—*Carnation streak virus.*—We have confirmed graft-transmission of carnation streak virus (Fig. 3) from carnation to carnation. In trials at Beltsville, a low percentage of carnation seedlings, grown adjacent to streak-affected plants in the open field during the summer and then moved to a greenhouse for further observation, developed streak symptoms. In spite of this indication of natural transmission of the streak virus, we have failed to find an insect capable of transmitting this virus in many trials, including those with the six-spotted leafhopper *Macrosteles fascifrons*.

In an attempt to confirm the claim of Thomas and Daniels (29) that streak virus is graft-transmissible from carnation to China aster, we attempted in May, 1955, to inarch China asters to King Cardinal carnation carrying streak, mosaic, and mottle viruses and to Virginia carnation carrying ringspot and mottle viruses. Five plants of American Branching Azure Blue aster were bound to King Cardinal and 6 to Virginia. The paired plants were left bound in contact for 9 weeks until the asters flowered and stopped growing. No symptoms of virus infection appeared in any of these 11 China asters. No evidence of union between the carnation and the China aster was detected. If these distantly related plants were capable of forming even a union of callus tissue, some transmission of the ringspot virus, to which China aster is susceptible, might be expected.

Aster yellows virus.—Thomas and Daniels (29) reported transmission by *Macrosteles fascifrons* of aster yellows virus from China aster to carnation, in which it produced streak symptoms, and also transmission of this virus from carnation to China aster. In earlier trials (24), we failed to transmit western aster yel-

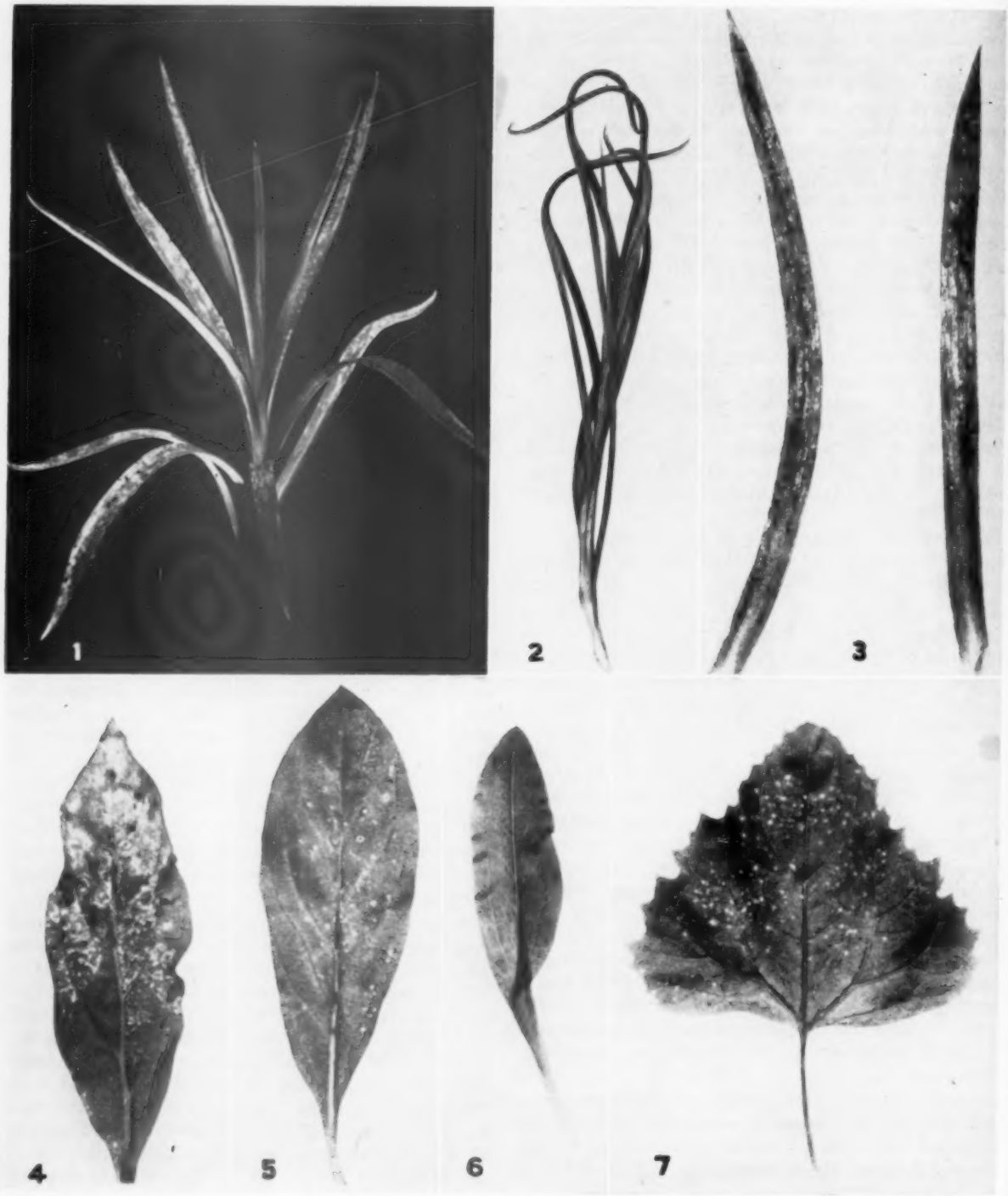

FIG. 1–7. FIG. 1. Feeding injury of the carnation aphid, *Myzus polaris*, in Puritan carnation.—FIG. 2. Acute symptoms of infection by western aster yellows in Donna Lee carnation.—FIG. 3. Carnation streak in King Cardinal carnation.—FIG. 4. *Gomphrena globosa* infected with carnation ringspot virus.—FIG. 5. *G. globosa* infected with carnation mottle virus.—FIG. 6. Carnation mosaic in *Dianthus barbatus*.—FIG. 7. *Chenopodium amaranticolor* infected with carnation mottle virus.

lows virus to carnation by means of this leafhopper vector. In March, 1956, we succeeded in transmitting the western aster yellows virus from *Vinca rosea* L. to Donna Lee carnation by dodder, *Cuscuta campestris* Yuncker. In shoot tips of this carnation, a distinctive inward curvature of leaves appeared (Fig. 2) after 20 weeks. *M. fascifrons* readily subtransferred the western aster yellows virus from infected Donna Lee carnation to China aster. The variety Donna Lee was selected for this test because naturally appearing symptoms in some plants suggested infection by aster yellows virus, but *M. fascifrons* did not transmit this virus from any plants of Donna Lee except those inoculated by dodder connection.

No streak symptoms, flower malformation, or grassy growth appeared in these carnations during the first 15 months after inoculation. Shoots with curled leaves (Fig. 2), rooted as cuttings, grew normally in our greenhouse and produced blooms normal in color and form. Evidently curling is a symptom of the acute stage of the disease only. *M. fascifrons* failed to transfer aster yellows virus from the recovered plants to China aster in March, 1957. This indicates that the virus is unable to maintain itself in Donna Lee carnation.

Carnation mosaic virus.—Carnation mosaic virus produces mottling in most commercial carnation varieties whenever the plants are in active growth. Conspicuous flower breaks are associated with this virus. Carnations naturally infected with the mosaic virus usually carry the more prevalent mottle virus and may carry carnation ringspot virus also. Such double or multiple infections account for many of the discrepancies in published reports.

Carnation mosaic virus is transmissible by sap and also by *Myzus persicae* in the nonpersistent manner. Aphid transmission separates this virus from the carnation mottle and carnation ringspot viruses. *Dianthus barbatus* is a distinctive test plant, producing vein mottling (17) after 8–10 days (Fig. 6) under favorable conditions and after about 21 days during dark periods of winter. A strong yellow mottle, often with green islands, develops later. This test reaction is not seriously disturbed by the presence of mottle virus or of ringspot virus.

Carnation mosaic virus produces mottling in *Dianthus chinensis* L., *D. deltoides* L., *D. latifolius* Willd., *D. plumarius* L., *D. superbus*, *Tunica saxifraga* (L.) Scop., and a species from Turkey received as *Dianthus giganteus*, in addition to the carnation itself and the test plant *D. barbatus*. *Silene compacta* Fisch. responds with chlorotic (King Cardinal strain) or light-brown spots (Peter Fisher strain), which become systemic but not lethal. Chlorotic local lesions develop in *Chenopodium album* and *C. amaranticolor* (12) and systemic red dot lesions in *Gomphrena globosa*. Symptoms in Chenopodium appear after 11–13 days; these are not surely recognizable in the presence of the earlier appearing local lesions caused by carnation mottle virus. The mosaic virus infects sugar beet (*Beta vulgaris* L. var. U. S. 401) without causing symptoms. It is not infectious to tobacco, bean, cucumber, or China aster.

Physical properties of mosaic virus were determined in December 1956 from unpurified extracts of *Dianthus barbatus* tested on this species and on *Chenopodium amaranticolor*. Two cultures of mosaic virus were tested, from the varieties King Cardinal and Peter Fisher, each collected in Oregon in 1945. Each virus was freed from mottle virus by transfer by means of *Myzus persicae*, but the possibility of latent virus accompanying the mosaic virus is not excluded. The virus from King Cardinal is more virulent to *D. barbatus* than is the one from Peter Fisher, and the 2 cultures differ in symptoms expressed in Chenopodium and Silene.

A period of dull cloudy weather followed inoculation in December. *D. barbatus* responded more slowly during this period than in summer, symptoms appearing after about 3 weeks. Chenopodium proved a poor indicator for the mosaic virus from Peter Fisher; it proved more sensitive than *D. barbatus* to the mosaic virus from King Cardinal in dilution tests but less sensitive in heating and aging tests.

The virus from King Cardinal was infectious after it was heated to 55° but not after it was heated to 60°C. It was infectious after dilution to 1–50,000 (Chenopodium) but not after aging for 6 days (*D. barbatus*). The virus from Peter Fisher was infectious after being heated to 50° but not after it was heated to 55°C. It was infectious after dilution to 1–100 but not after aging 6 days.

Physical properties of the mosaic viruses were rechecked in March, 1957, with extracts of *D. barbatus* tested on this species, on *D. superbus*, and on *Chenopodium amaranticolor*. The virus from King Cardinal was infectious after being heated to 60° but not when heated to 65°C; *D. barbatus* and *D. superbus* gave the same end point, but the test on Chenopodium was positive with extract heated at 50° and negative with juice heated at 55°. This virus was infectious to Chenopodium at dilution of 1–100,000, to *D. superbus* at 1–50,000, and to *D. barbatus* at 1–5000. On aging at 20°C, the virus was infectious to *D. superbus* after 8 days (not after 10 days), to *D. barbatus* after 4 days, and to Chenopodium after 2 days.

The mosaic virus from Peter Fisher was infectious to *D. barbatus* and *D. superbus* after it was heated to 55° (not after being heated at 60°), to *D. superbus* after dilution to 1–5000, to *D. barbatus* after dilution to 1–1000, and to both species after aging 6 days (not after 8 days). Again, Chenopodium proved insensitive to the virus from Peter Fisher, but it was more sensitive than the other indicator plants to the virus from King Cardinal in dilution tests, less sensitive in heating and aging tests. Evidently dilution frees this virus from an inhibitor present in *D. barbatus* (30). *D. superbus* is superior to *D. barbatus* as an indicator for carnation mosaic virus, but it fails to distinguish sharply between this virus and mottle virus. Also,

seed of *D. superbus* of sufficient uniformity for test purposes is often difficult to obtain.

Since the mosaic virus has been confused with the mottle virus as well as with carnation ringspot virus, some published host records properly apply to these other viruses. It is not practicable to recheck all hosts recorded by Gasiorkiewicz (8) and by Thomas (25). The local-lesion response after 7 days in *Chenopodium album* reported for carnation mosaic by Gasiorkiewicz (9) is actually a response to the carnation mottle virus. Strains of carnation mosaic virus reported by Thomas (27) may include strains of carnation mottle virus. There is evidence of strain differences in both these viruses, but the role of Kassanis' latent (15) virus in influencing intensity of symptoms in *D. barbatus* in the United States is not known. It seems likely that the rod-shaped particles derived from mosaic-affected carnations by Johnson (13) were those of carnation latent virus, for Kassanis (17) found rod-shaped particles associated with this virus. No facilities are available to us for detecting the carnation latent virus, which is serologically active and visible in electron micrographs.

Carnation mottle virus.—Carnation mottle virus (17) is common in commercial carnations, in which it produces either faint leaf-mottling or no symptoms. It causes flower breaks in Perpetual Giant seedlings but only faint streaking, chiefly on the backs of the outermost petals, in red and pink commercial varieties. This virus causes distinctive local lesions in *Chenopodium amaranticolor* (Fig. 7) and *C. album* after 6–7 days and mild mottling in *Dianthus barbatus* after 10–12 days. The reaction in Chenopodium is distinctive and is not disturbed by the presence of mosaic virus in the inoculum. Most of the yellow local lesions caused by the mottle virus become red rings with gray centers after 2 or more weeks. These local lesions are produced abundantly and usually are distinguishable in the presence of lesions caused by carnation ringspot virus, which appear 2 days earlier. Carnation mottle virus infects also *Gomphrena globosa* (Fig. 5), *Silene compacta*, and *Dianthus superbus* but not sugar beet, tobacco, or bean.

Many isolates of mottle virus, although free from ringspot virus, produce blotches or ring symptoms in *D. barbatus*. The isolate from Light Pink Littlefield, which we selected for study, causes mild mottling only in *D. barbatus*. The mild mottling is confined to young leaves, which become symptomless as they mature, so that mottle symptoms seem to disappear and then reappear.

Properties of carnation mottle virus were determined in October, 1956, with unpurified extracts of *Dianthus barbatus* tested on *Chenopodium amaranticolor*. This virus was infectious after being heated for 10 minutes at 85°C (not after being heated to 90°), after dilution to 1–5,000,000, and after aging at room temperature (about 20°) for 18 days (not after 21 days). Eight local lesions appeared on 3 test plants after dilution to 1–5,000,000; the identity of these lesions was confirmed by subtransfer. Chenopodium is more sensitive than *D. barbatus* as an indicator for this virus.

Carnation ringspot virus.—Carnation ringspot virus, described as anjermosaiek virus by Noordam and coworkers (21), is called carnation ringspot virus in England (17) and in America (5). This is evidently the virus reported by Wright (32) as causing local lesions in *Gomphrena globosa* 5 days after inoculation. So far as we know, this virus is relatively rare in the United States. We isolated it from 1 of 30 commercial carnations indexed in 1946, the variety Virginia from Spokane, Washington. Samples were later submitted to us by G. A. L. Mehlquist from Missouri and by C. J. Olson from Ohio.

Carnation ringspot virus is not transmitted by *Myzus persicae;* no vector is known. Thirty species of plants proved susceptible in our tests; 30 others failed to respond to sap inoculation. Systemic infection developed in *Arenaria montana* L., *Celosia cristata* L., *Chenopodium album*, *C. amaranticolor*, *Dianthus barbatus*, *D. caryophyllus*, *D. superbus*, *Gomphrena globosa*, *Nicotiana tabacum* L., *Phaseolus vulgaris* L., and *Silene compacta*. Local lesions, from which the virus was recovered, appeared in *Antirrhinum majus* L., *Callistephus chinensis*, *Cucumis sativus* L., *Datura innoxia* Mill., *D. stramonium* L., *Dianthus arenarius* L., *D. caesius* Smith, *D. deltoides*, *D. plumarius*, *Lathyrus odoratus* L., *Saponaria ocymoides* L., *Spinacea oleracea* L., and *Ulmus americana* L. Other plants expressed no symptoms following inoculation with carnation ringspot virus, but this virus was recovered on subinoculation to Gomphrena from the following: *Althaea rosea* Car., *Beta vulgaris*, *Calendula officinalis* L., *Glycine* Max (L.) Merr., *Tagetes patula* L., and *Tunica saxifraga*.

Van der Want (30) pointed out that carnation ringspot virus is difficult to transmit from carnation to aster, to tobacco, and to bean but that Gomphrena serves as a bridging host (31). In tobacco, the symptoms caused by carnation ringspot virus are strictly local, but we have succeeded in isolating the virus by subinoculation to Gomphrena from symptomless upper leaves and from roots. The low concentration in the symptomless upper leaves of tobacco 28 days after inoculation is sufficient to protect such leaves against superinfection by carnation ringspot virus; however, the viruses of tobacco ringspot, tomato ringspot, alfalfa mosaic, and cucumber mosaic can be superimposed in such plants, which then show symptoms typical of infection by the respective viruses. Carnation ringspot virus is readily sap-transmitted to and from American elm in which it develops brown local lesions unlike those induced by the naturally occurring ringspot viruses in elm, which are at least partly systemic. The lethal effect on *Silene compacta* is a distinctive reaction to the carnation ringspot virus.

Physical properties of carnation ringspot virus determined in November, 1956, with extracts prepared from *Dianthus barbatus* and tested on *Chenopodium amaranticolor* and on *Gomphrena globosa*, are as follows: thermal inactivation point (10 minutes), 80–

85°; dilution end point, between 1–100,000 and 1–500,000; and aging at room temperature (about 20°C), between 16 and 20 days. The test plants Chenopodium and Gomphrena yielded the same end points, but lesions in Gomphrena are more distinctive than those in Chenopodium.

Symptoms of the acute stage of carnation ringspot in leaves of Perpetual Giant carnations are gray rings; symptoms of the chronic stage include irregular chlorotic spots and occasional chlorotic ring patterns. The commercial carnation varieties King Cardinal, Louisa, and Virginia show irregular gray or yellow spots and streaks in leaves. Some leaf distortion accompanies these lesions; leaf margins are frequently waved or otherwise irregular.

Symptoms induced by carnation ringspot virus in Gomphrena (Fig. 4) include gray rings that appear after 4–5 days, yellowish mottling that soon follows, and red rings that appear after 10–14 days. These red rings resemble those produced by the mottle and mosaic viruses, both in appearance and in timing; however, our carnation ringspot virus culture still induced these symptoms after it was subinoculated from Gomphrena, after it was heated to 75°C, after it was diluted to 10^{-5}, after reisolation from symptomless upper leaves of infected tobacco, and after reisolation from American elm. We therefore consider that the mottling and red-ring symptoms in Gomphrena are authentic expressions of infection by the carnation ringspot virus. Symptoms induced by carnation ringspot virus in *Chenopodium amaranticolor* are solid brown local lesions that appear after 4–5 days; these later enlarge and cause leaf distortion and leaf fall. Similar systemic lesions follow. When freed from the mottle and mosaic viruses, the ringspot virus no longer produces mottling in *D. barbatus;* only irregular plotches and occasional rings develop during the chronic stage of the disease.

Prevalence of viruses in American carnations.—In April, 1955, on examining 151 carnation varieties at Beltsville for symptoms of carnation streak, we found 11.2 per cent of them infected; these represented stocks from 9 growers and from 4 states. During May, June, and October, 1955, we indexed these 151 varieties by sap inoculation to *Dianthus barbatus* var. Holborn Glory and to *Gomphrena globosa* var. Rubra. The samples were ground in 0.1 M K_2HPO_4, and the test plants were washed 15–20 minutes after inoculation. Carnation ringspot virus was found in only 2 varieties (1.3 per cent) from 2 growers in Massachusetts. Carnation mosaic virus was detected in only 5 varieties (3.3 per cent) in tests on *D. barbatus.* Carnation mottle virus was found in 139 varieties (92 per cent), representing all state sources and including the leading modern varieties of the Sim and Littlefield classes. The fact that no virus was detected in 10 random samples of R. N. Stewart's 1-year-old seedlings that had not yet been propagated indicates that seed transmission of viruses in carnation is lacking or infrequent.

Root transmission of carnation mottle virus.—Thomas and Baker (28) reported transmission of carnation mosaic virus by root contact. In October, 1955, healthy seedlings of *Dianthus barbatus* were transplanted to 8-in. pots together with carnations carrying one of the following viruses or virus mixtures: 1) mottle virus; 2) mosaic, streak, and mottle viruses; and 3) ringspot, mosaic, and mottle viruses. There were 15 pots in all; test seedlings were paired with each source type in each of 5 pots. No barriers were placed between the paired plants, and the roots and tops of the test plants of *D. barbatus* were allowed to mingle freely with those of plants carrying the carnation viruses.

After 6 months' contact, no symptoms were recognized in *D. barbatus* by direct inspection. In April, 1956, the 15 test plants of *D. barbatus* were indexed individually by sap inoculation to 2 plants of Chenopodium and to 2 of Gomphrena. The carnation mottle virus was recovered from 1 plant of those paired with a plant infected with the mottle virus. Thus, transmission of the highly infectious carnation mottle virus by contact of roots and tops is much less probable than is transmission by handling practices such as the taking of cuttings by knife.

Cutting-knife transmission of mottle and ringspot viruses.—Since carnation mottle virus proved so common in American carnations and since it is not aphid borne, we suspected that it might be carried by some handling practice in use in commercial carnation culture. Therefore an experiment on transmission by the cutting knife was carried out. The source plants were of the King Cardinal variety collected in Oregon in 1945; they were naturally infected with streak, mosaic, and mottle viruses. In addition, 3 of these plants were experimentally inoculated with carnation ringspot virus from a culture obtained from the variety Virginia from Washington in 1945.

Test plants were Perpetual Giant seedlings from seed sown in November, 1955. An indexing of these seedlings in March, 1956, revealed no virus present. These garden-type carnations were chosen because of the difficulty of finding strictly virus-free commercial-type stock. Cuttings taken from these seedlings with a flamed knife were exposed in April, 1956, by passing a knife through a young shoot of the King Cardinal source plant immediately before passing it through the base of each test cutting. These cuttings were then rooted in sand and later potted and held in an isolated area in a fumigated greenhouse until September, 1956, when they were indexed.

Each of 73 seedlings was indexed by sap transfer from a young vegetative shoot to 2 plants each of *Chenopodium amaranticolor, Dianthus barbatus,* and *Gomphrena globosa.* The appearance after 4–5 days of local lesions in all 3 test species was considered evidence of the recovery of carnation ringspot virus. Yellow-dot local lesions in Chenopodium after 6–7 days indicated recovery of carnation mottle virus. Carnation mosaic virus was not recovered from any of

the test plants. Ringspot virus was recovered from 19 plants (26 per cent) and mottle virus from 45 plants (61.6 per cent).

In October, 1956, the Perpetual Giant carnation stock plants that supplied the cuttings used as test plants in the cutting-knife transmission experiment were again indexed, but no virus was found in them. These plants were held on another bench in the same greenhouse that was used in the cutting-knife test.

Discussion.—The name "carnation mosaic virus" has long been in use in the United States and should have priority over Kassanis' (17) "vein mottle virus" with which it is in fair agreement. The strains of carnation mosaic virus we studied are more tolerant of heat than that described by Kassanis, but there is evidence of strains milder than ours; these might well conform closely to the virus studied by Kassanis. Kassanis' (17) name "carnation mottle virus" should stand because Kassanis first recognized this mild and prevalent virus as a separate entity.

Gomphrena is susceptible to mosaic, mottle, and ringspot viruses. The red dots or red rings, at first 0.5 mm in diameter but finally enlarging to 2 mm or occasionally to 4 mm, appear in Gomphrena 10–14 days after inoculation with each of these 3 carnation viruses. The similarity in appearance and timing of these lesions suggested that yet another virus entity might be involved. We suspected that these lesions might represent a response to the carnation latent virus of Kassanis (17), since Köhler (18) reported an aberrant form of potato S virus infectious to Gomphrena. The carnation latent virus and potato S virus show serological relationship but are not identical (17); however, D. H. M. van Slogteren of the Laboratorium voor Bloembollenonderzoek, Lisse, The Netherlands, who kindly examined carnation samples carrying mottle virus that causes the red-ring reaction in Gomphrena, found no reaction with potato S virus antiserum and no rod-shaped particles in electron micrographs.

It was found difficult to subculture virus to Chenopodium from the small red rings with gray centers (Fig. 5) that appear in Gomphrena as symptoms of mottle virus infection, but eventually we succeeded in this subtransfer. The red rings in Gomphrena are therefore acceptable as symptoms of infection by carnation mottle virus. Carnation mosaic virus is readily transferred from the solid red dots in Gomphrena to other plants of Gomphrena, but difficult to transfer from Gomphrena to *D. barbatus*. This transfer also finally succeeded, confirming the red dots in Gomphrena as symptoms of infection by carnation mosaic virus. Virus is easily subcultured from the red rings produced in Gomphrena by carnation ringspot virus, and such transfers reproduce in Gomphrena the usual sequence of gray rings, yellowish mottling, and finally red rings. The red-dot symptoms in Gomphrena are readily produced by sap transfer of mosaic virus from *D. barbatus* that had been inoculated by means of *Myzus persicae*; no reaction in Gomphrena was produced by sap transfer from *D. barbatus* that had been inoculated with mottle or ringspot virus by means of *M. persicae*.

Transmission of carnation viruses to sugar beet is difficult. Sap transfer of ringspot virus from Gomphrena in April failed, but similar transfer from *D. barbatus* in November succeeded. No symptoms appeared in the inoculated beets, but the virus was recovered in Gomphrena. Sap transfer of mosaic virus from *D. barbatus* to beet succeeded in January and sap transfer from King Cardinal in December; mosaic virus was recovered from the symptomless beets. All attempts to transmit carnation mottle virus to sugar beet have failed. Two of the beets inoculated by sap from King Cardinal expressed dull yellowing in rubbed leaves, suggestive of the latent virus reaction reported by Kassanis (17). Also, the King Cardinal mosaic virus was recovered from sugar beet in undiminished virulence, whereas it is weakened in virulence after aging for 8 days.

Visual inspection indicates that American commercial carnations carry much less virus infection than they did 10 years ago. This may be attributed to increase in the practice of year-round greenhouse culture of carnations and to use of mother-blocks as sources of cuttings. These measures have been adopted chiefly to control Alternaria blight, bacterial wilt, and Fusarium wilt. They have also served to control streak and mosaic, since these virus diseases are recognizable in well-grown plants and tend to be eliminated from mother blocks. The mottle virus, however, produces unreliable symptoms, or none, in commercial carnations and can be detected only by indexing. This virus is so prevalent and so readily transmitted in the course of handling the crop that there is no prospect that it can be controlled by any method short of revamping the entire carnation industry. No evidence is available as to what damage the mottle virus causes in commercial carnations. The American commercial grower will presumably be content to eliminate the diseases he can see and to live with those that remain unrecognized.

The high frequency of transmission of mottle virus on the cutting knife in our experiment explains the prevalence of this virus in commercial carnations. It is true that the conditions of our test were more severe than would be encountered in commercial propagation, and also our test seedlings may be more susceptible to the mottle virus than are most commercial sorts. Nevertheless the carnation mottle virus is clearly one of the more infectious plant viruses, and cutting the blooms from commercial carnations must surely effect some transmission.

Transmission of carnation ringspot virus by the cutting knife was not anticipated. The ready transmission of this virus in our experiment would suggest that this virus should be more prevalent in American commercial carnations than we have found it to be; however, this virus is heat-sensitive, as Kassanis (16) showed. The titer may therefore be low in summer

and perhaps also lower in commercial sorts than in the seedlings used in our transmission experiment. It is doubtful whether the chronic symptoms of carnation ringspot are sufficiently well defined in our carnation varieties to permit efficient roguing of affected plants from mother blocks. Spring is the best season for indexing carnations, for Gomphrena may fail to detect ringspot virus during hot weather and *Dianthus barbatus* may fail to detect mosaic virus during dark winter days.

Some commercial growers now break the cuttings from the stock plants and refrain from trimming cuttings, chiefly to avoid transmission of the bacterial wilt organism. This practice should, of course, help to reduce transmission of the more infectious carnation viruses. The mosaic virus, in contrast, seems dependent on aphid transmission, a hazard that is greatly reduced by the modern practices of continuous greenhouse culture and efficient fumigation.

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SOME CHEMICAL PROPERTIES OF PHENYLMERCURY ACETATE IN RELATION TO FUNGICIDAL PERFORMANCE[1]

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SUMMARY

Phenylmercury acetate and ethylmercury chloride were removed from dilute aqueous solution by soils, especially organic types. This loss may be prevented by formulation with thiols or cyanide.

Dilute phenylmercury acetate solutions are weakened by contact with aluminum, iron, or wood but not by contact with stainless steel, brass, copper, or concrete. Small amounts of sodium hydroxide improved the stability of phenylmercury acetate solutions to iron, and an equivalent amount of thiomalic acid and buffering at pH 10.5 improved the stability to wood.

Formulation of phenylmercury acetate with thiol acids and buffering at pH 10.5 promoted the formation of diphenyl mercury, a relatively inert material in laboratory tests.

Phenylmercury acetate formulated with thiol acids at pH 10.5 appeared to be as effective in control of basal rot of narcissus as did the unformulated solution, provided concentrations sufficiently high to leave an equivalent amount of phenylmercury acetate on the bulb were used.

Phenylmercury acetate (PMA)[2] is the fungicide now generally used commercially for controlling basal rot of narcissus caused by *Fusarium oxysporum* Schw. f. *narcissi* Snyd. & Hans. (3). In field use of PMA to control basal rot, the bulbs are first sorted, cleaned, and placed in wooden flats. The flats containing the bulbs are then dipped in large concrete or metal tanks containing the fungicidal solution. The tanks may have agitators and heaters. Thus the fungicidal solution may be in contact with several types of metal, with the bulbs, with wooden flats, and with soil not removed from the bulb in the cleaning process. This report presents the results of an investigation of the chemical properties of PMA as they affect fungicidal performance in control of basal rot of narcissus.

SOLUBILITY OF PHENYLMERCURY ACETATE.—Information on the solubility of PMA in common solvents is incomplete; therefore, solutions of approximate saturation were prepared by shaking an excess of PMA with a solvent for 20 hours at room temperature. The resulting solution was centrifuged, and a weighed amount of the supernatant liquid was diluted, acidified with nitric acid, and titrated within 1 or 2 days with standard thiocyanate as described for silver (4). PMA dissolved readily in many solvents, including common organic solvents, acetic acid, concentrated phosphoric acid, and dilute ammonia (Table 1). It was least soluble in water.

SOIL EXPERIMENTS.—From preliminary experiments (8) it appeared that soil was the main cause of decreased fungicide concentration in bulb-treating solutions. Therefore, a standardized test was developed to determine the effect of soil on PMA formulations.

A 1-g sample of soil was weighed into a 125-ml Erlenmeyer flask. Fifty ml of the aqueous fungicidal formulation under test was added, and the flask was swirled a number of times during a 48-hour standing period. The original concentration of the fungicide solution (150–200 ppm) and the final concentration were determined by methods previously described (6, 7).

The soils used in the experiments were those in which narcissus bulbs are grown in various parts of the United States. The soil samples were screened through a 32-mesh screen. Rocks, roots, and other extraneous material were discarded.

The soils differed greatly in their ability to remove PMA from solution (Table 2). Peat soil was particularly destructive. In general, the unformulated PMA (approximately neutral in reaction) was the most readily adsorbed by soil. Most commercial formulations tested were in this category (cf. Tag in Table 2). When the pH of the solution was lowered to pH 1.5–2.8 or increased to pH 8.8–10.5, the effect

TABLE 1.—*The approximate solubility of phenylmercury acetate (PMA) in several solvents at room temperature*[a]

Solvent[b]	Solubility (g/100 g solution)	Solvent[b]	Solubility (g/100g solution)
Diethanolamine (50%)	62	2-Methoxyethanol	16
Ethanolamine	57	Ethanolamine (6.4%)	16
Pyridine	50	Acetic acid (75%)	15
Trifluoroacetic acid	45	Na-Versenate[c] (30%)	12
Triethanolamine (50%)	40	Dihydropyran	12
Pyrrolidine	36	Echridine[d]	12
Ethanolamine (50%)	28	Ammonia (1.75%)	11
Pyridine (50%)	27	Tetrahydropyran	11
Pyrrolidine (50%)	26	Acetonylacetone	10
Acetic acid	25	Diacetone alcohol	9
Triethanolamine	19	Phosphoric acid (85%)	8
Diethylamine	19	Water	0.45
Allyl alcohol	18		
Morpholine	16		

[a] An excess of PMA was shaken in the solvent for 20 hours.

[b] Pure solvents were used unless otherwise indicated; the exceptions were aqueous solutions (volume/volume basis).

[c] Ethylenediamine tetraacetic acid.

[d] 3(4'Ethylcyclohexylmethyl) pyridine.

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[2] Refers to Mersolite 8 (97.5% phenylmercury acetate) unless otherwise stated.

TABLE 2.—*Removal of fungicides from 50 ml aqueous solutions containing 150–200 ppm active ingredient by 1 g of soil in 48 hours*

Fungicide and formulation	pH	Percentage fungicide remaining after exposure to indicated soil[a]								
		1	2	3	4	5	6	7	8	9
Phenylmercury acetate (PMA) unformulated	5.0	25	53	69	29	32	2	36	29	18
Tag[b]	7.7	27	66	76	45	44	2	36	27	20
PMA + thiomalic acid[c]	4.0	86	93	92	90	93	75	94	90	86
PMA + thiomalic acid[c] + Na_2CO_3	10.5	99	100	99	99	99	94	99	99	99
PMA + NaOH (0.02N)	11.9	88	95	97	93	92	25	99	87	84
PMA + acetic acid (0.15 N)	2.8	72	87	94	70	68	9	86	77	62
PMA + NaCN[c] + Na_2CO_3	10.5	100	100	98	96	98	80	98	98	100
Ethylmercury phosphate (unformulated) + NaCN[c] + Na_2CO_3	7.2	49	81	88	65	59	5	78	53	41
	10.5	95	95	94	98	95	92	97	96	93
Ethylmercury chloride[d]	11.7	83	96	98	93	90	10	94	90	82
Cyano (methylmercuri) guanidine[e]	7.2	61	92	97	77	68	9	78	57	45

[a] The soils used were as follows: 1) loam from Long Island, New York; 2) sandy loam from USDA Narcissus Plots, Beltsville, Md.; 3) loamy sand from Beltsville, Md.; 4) fine sand from the area around Wilmington, N. C.; 5) very fine loamy sand from near Wilmington, N. C.; 6) peat soil from Puyallup, Wash.; 7) sandy loam from Puyallup, Wash., the principal bulb-growing soil of the area; 8) silt loam from Clark County, Wash.; 9) silty clay loam from Puyallup, Wash. Soils 1 through 5 obtained from W. D. McClellan, USDA, Beltsville, Md.

[b] Commercial formulation containing 10% phenylmercury acetate.

[c] Thiomalic acid and sodium cyanide were added according to the equations:

$$RHgOAc + R'SH \rightarrow RHgSR' + HOAc.$$

$$RHgOAc + 2NaCN \rightarrow NaRHg(CN)_2 + NaOAc.$$

[d] Used as 2% Ceresan.

[e] Used as Panogen (2.2% active ingredient).

of soil is decreased. This is represented by 0.15 N acetic acid in Table 2, since the effects of both pH changes were very similar with respect to loss of PMA in contact with soil. At pH 12 (0.02 N NaOH in Table 2), the effect of soil was further diminished. PMA is not stable in the presence of strong mineral acid.

The effect of acid and alkali may be explained by the suppression of phenylmercury ions in the presence of large amounts of hydrogen or hydroxyl ions. With strong alkali, a salt somewhat similar to sodium phenolate is formed. The maximum stability of PMA solutions was obtained by formation of complex compounds that did not permit ionization of PMA as such. When a thiol compound with an organic acid group is used, a sodium salt will be formed with sodium carbonate, with ionization according to the equation

$$RHgSR'COONa \rightleftharpoons RHgSR'COO^- + Na^+.$$

PMA solutions of stability comparable to that formulated with thiomalic acid at pH 4.0 (Table 2) may be prepared with thioglycollic acid, thioethanol, or monothioglycerol. Somewhat better soil stability was obtained with alkaline sodium cyanide; however, there is no evidence that the complex cyanide $NaRHg(CN)_2$ was formed, since pure synthesized phenylmercury cyanide was equally effective in slightly acid (approximately pH 5) solution. Thiol acid formulations at pH 10.5 provided maximum soil stability. Results comparable to those obtained with thiomalic acid and sodium carbonate (Table 2) were obtained with thioglycollic acid, β-isothiouriedopropionic acid, and thiosalicylic acid. The behavior of ethylmercury phosphate and that of cyano(methylmercuri)guanidine toward soil were very similar to that of PMA.

STABILITY OF PMA SOLUTIONS IN CONTACT WITH METAL AND OTHER MATERIALS.—During the dipping of bulbs, the PMA solution may contact wood, concrete, and/or various metals. Therefore, an experiment was set up to determine the effect of these materials on the stability of PMA solutions. One hundred ml of solutions of PMA (100–200 ppm) was placed in each of several wide-mouth 250-ml Erlenmeyer flasks. Strips of metal sheet (approximately 100 × 30 mm and sanded to remove surface oxide), small broken pieces of concrete, or wood chips were placed in the flasks. The flasks were stoppered and allowed to stand for 2 weeks with occasional swirling.

The results indicate that unformulated PMA solutions may be handled in contact with stainless steel, copper, brass, or concrete without loss (Table 3). Severe loss occurs with aluminum, wood, or iron. PMA in 0.02 N sodium hydroxide was not weakened appreciably by contact with stainless steel, iron, copper, brass, or concrete, but lead or aluminum destroyed it almost completely. The formulation with thioglycollic acid at pH 4 was satisfactory only with stainless steel. The thiomalic acid formulation showed stability in the presence of stainless steel, copper, brass, lead, concrete, or wood. The stability to iron was good in strongly alkaline sodium hydroxide, moderate at pH 10.5, and poor in the essentially neutral solutions. Thioethanol-stabilized solutions were satisfactory with stainless steel or concrete. Therefore, it appears that during the usual bulb-treating procedure, the concentration of the fungicidal solution may be lowered by the concrete tanks, wooden crates, and iron in the equipment, as well as by the soil on the bulbs.

TEST FOR FUNGICIDAL EFFICIENCY.—Repeated spore

TABLE 3.—*The stability of 100- to 200-ppm aqueous phenylmercury acetate (PMA) solutions exposed to metals, wood, or concrete for a 2-week period*

Formulation	pH	Percentage fungicide remaining in solution after exposure to indicated material[a]								
		1	2	3	4	5	6	7	8	9
Unformulated PMA	4.6	100	62	23	1	100	99	89	100	28
PMA + 0.02 N NaOH	12.0	100	79	95	1	97	100	2	100	57
PMA + Thioglycollic acid[b]	4.0	99	60	2	11	13	63	64	5	37
PMA + Thiomalic acid[b] + Na_2CO_3	10.5	100	50	71	0	96	100	100	98	95
PMA + Thioethanol[b]	5.0	100	80	5	2	84	83	82	93	83

[a] Materials tested were as follows: 1) stainless steel; 2) galvanized iron; 3) ordinary iron; 4) aluminum; 5) copper; 6) brass; 7) lead; 8) concrete; and 9) wood.
[b] Thiol compounds were formulated with PMA according to the equation:
$RHgOAc + R'SH \rightarrow RHgSR' + HOAc$.

germination tests with the basal rot organism *Fusarium oxysporum* f. *narcissi* were made. Results indicated that any of the 4 compounds phenylmercury acetate, ethylmercury phosphate, ethylmercury chloride, or cyano(methylmercuri) guanidine was much superior on an equivalent mercury basis to diphenyl mercury, diethyl mercury, or mercuric chloride. In these same tests, unformulated preparations of PMA, ethylmercury chloride, and methylmercury chloride were compared with preparations of the respective materials formulated with such stabilizers as sodium hydroxide, sodium thiosulfate, thiomalic or other thiol acids, sodium cyanide, or thioethanol. Some acid formulations were buffered at pH 10.5. Repeated spore germination tests at 9 equivalent mercury levels failed to show consistent superiority of any formulation or of the unformulated mercurial under the conditions of these tests. Sodium thiosulfate in PMA formulations has been reported to affect adversely bacteriocidal action (5) and chemotherapeutic index (1). These investigators, however, used other organisms; this may explain the discrepancy. Also, it is possible that the relatively small amount of thiol compound present in our experiments was oxidized by air, regenerating the original mercurial.

Consideration was given to the possibility of the formation of diphenyl mercury, an inferior fungicide, according to the equation

$$2RHg^{+} \leftrightharpoons R_2Hg + Hg^{++}.$$

The elimination of the Hg^{++}, such as by the formation of mercuric sulfide, would favor the reaction's proceeding to the right. Therefore, tests were made for the effect of all stabilizers on the formation of diphenyl mercury.

Under the usual conditions of treatment, the fungicide dries on the bulbs. Therefore, to simulate the normal bulb treatment, various formulations of PMA were allowed to dry on petri dishes for 48 hours. The dried material was extracted first by chloroform and then by water. The chloroform solution was analyzed for diphenyl mercury and for PMA and the aqueous solution for PMA by a procedure published elsewhere (7).

The tendency to form diphenyl mercury was slight with unformulated PMA, with PMA plus thiol acids at pH 6.5 or less, or with PMA in sodium hydroxide solution at pH 12 (Table 4). Similarly, PMA formulated with the neutral thiol compounds thioethanol and monothioglycerol was converted to diphenyl mercury in the range of 10 per cent (not shown in Table 4). When PMA was formulated with thiol compounds at a pH greater than 7.5, the tendency to formation of diphenyl mercury was increased somewhat by thiomalic acid, considerably by sodium thiosulfate or thiosalicylic acid, and greatly by thioglycollic acid. When sodium sulfite was added to prevent oxidative decomposition of thioglycollic acid, an even larger amount of diphenyl mercury formed. A possible explanation for these observations may be related to the stability of the acid, since thioglycollic acid is less stable than thiomalic acid.

Diphenyl mercury was very readily converted to phenylmercury compounds in the presence of soluble mercuric compounds and moisture. When calculated quantities of aqueous mercuric chloride were added to dry diphenyl mercury, phenyl mercuric chloride was formed almost quantitatively. Even use of mercuric sulfide as the mercuric compound resulted in appreciable (10–25 per cent) diphenyl mercury being converted to a phenylmercury compound. Repeated

TABLE 4.—*Percentage of phenylmercury acetate (PMA) converted to diphenyl mercury when different formulations containing 400 μg PMA were allowed to dry on petri dishes*

Formulation[a]	pH	Percentage converted	Trials (triplicate)
Unformulated PMA	5.0	9	3
PMA + thiomalic acid	4.0	4	1
PMA + thioglycollic acid	4.0–6.5	9	6
PMA + sodium hydroxide (0.02 N)	12.0	10	2
PMA + thiomalic acid + Na_2CO_3	10.5	21	3
PMA + sodium thiosulfate	10.5	43	2
PMA + thiosalicylic acid	10.5	47	5
PMA + thioglycollic acid	7.5–10.5	65	5
PMA + thioglycollic acid + Na_2SO_3 (0.1%)	10.5	82	2

[a] PMA formulated according to this equation:
$RHgOAc + R'SH \rightarrow RHgSR'$.

attempts to detect diphenyl mercury on bulb husks treated with either formulated or unformulated PMA were unsuccessful, although added diphenyl mercury was recovered satisfactorily.

EXPERIMENTS WITH NARCISSUS HUSKS.—In preliminary experiments, it was found that 70 per cent or more of the PMA on treated narcissus bulbs was on the husks. Most of the remainder was on the basal plates. Husks were used therefore, to represent the whole bulb in experiments on the adsorption of fungicide from various formulations. Two g of husks were used per 150 ml of 250-ppm PMA solution. The standard dipping time was 5 minutes. The results indicate that formulations that prevent PMA from being adsorbed by soil also reduces the amount of the fungicide remaining on the bulb. Thus, husks dipped in unformulated PMA (250 ppm) contained 5000 ppm; those dipped in PMA formulated with sodium hydroxide contained 1400 ppm; and those dipped in PMA formulated with thiomalic acid at pH 10.5 contained only 400 ppm PMA. Similarly, husks were dipped in various concentrations of unformulated PMA, with time held constant. When the amount adsorbed from 250-ppm solutions of unformulated PMA is referred to as 100 per cent, the amounts adsorbed from 150-ppm, 50-ppm, and 10-ppm solutions were 55, 17, and 4 per cent, respectively. Thus the amount of PMA adsorbed was roughly proportional to the concentration of the PMA solution. Also, when husks were dipped in unformulated 250-ppm PMA solution for various times, the husks adsorbed 2000 ppm in the first half minute, 3900 ppm in 3 minutes, 5600 ppm in 5 minutes, 7300 ppm in 10 minutes, and 9800 ppm in 60 minutes.

EXPERIMENTS WITH NARCISSUS BULBS.—Englehart narcissus, a variety very susceptible to basal rot, was used to determine the effectiveness of PMA in formulations. Five hundred bulbs per treatment were dipped in a suspension of spores of *Fusarium oxysporum* f. *narcissi* and stored for several days under warm, moist conditions favorable to growth of the fungus. The bulbs were dipped in 200-ppm PMA solutions, unformulated, and formulated with sodium hydroxide and with sodium cyanide at pH 10.5. Similarly formulated dips containing 300 ppm PMA were also used. For comparison, 2% Ceresan (2% ethylmercury chloride) was used as a dip at 300 ppm active ingredient. At 200 ppm PMA, approximately 25 per cent of the bulbs were rotted regardless of formulation. At 300 ppm PMA approximately 20 per cent of the bulbs were rotted in all treatments. Forty-three per cent of the check bulbs were rotted. The data indicate that the formulations used had no detrimental effect on fungicidal effectiveness.

Data from further investigations in 1954 of the effect of formulations on fungicidal efficiency are reported in Table 5. Unformulated PMA appeared somewhat better than did the fungicide plus thioglycollic and/or thiosalicylic acid at pH 10.5 in tests on the variety Golden Harvest; however, in the tests on the variety Spring Glory, unformulated PMA was no better than the formulations.

TABLE 5.—*Percentage of narcissus bulbs that rotted following inoculation with Fusarium oxysporum f. narcissi and subsequent dip treatment with various formulations of phenylmercury acetate (PMA)*

Formulation			Percentage of rotted bulbs in indicated variety[b]	
PMA (ppm)	Stabilizer[a]	pH	Golden Harvest	Spring Glory
None			68	24
240	None	5.0	7	5
240	Thioglycollic acid	4.0	14	
240	Thiosalicylic acid + Na_2CO_3	10.5	21	
240	Thioglycollic acid + Na_2CO_3	10.5		4
240	Thioglycollic acid + Na_2CO_3 + Na_2SO_3 (0.1%)	10.5		5
240	Thiomalic acid	4.0		4
240	Thioethanol	5.0		6

[a] Stabilizers added according to the formula:
$RHgOAc + R'SH \rightarrow RHgSR' + HOAc$.
[b] Based on inoculated bulbs: 500 Golden Harvest or 250 Spring Glory.

The data obtained when husks were dipped in formulated or unformulated PMA and the results of the preceding experiments indicate that the amount of fungicide remaining on the bulb may influence the amount of protection afforded. It was not found possible, however, to prepare formulated and unformulated solutions that would deposit the same amount of PMA on the bulb because of variability in the bulbs. In 2 such attempts, bulbs of the variety Golden Harvest were inoculated, dipped, and stored as previously described. One lot consisted of freshly dug bulbs of high quality and the other of bulbs of poorer quality that had been dug several weeks previously. The difference in percentage of rotted bulbs in the check group in the 2 trials (Table 6) is not surprising, since the bulbs become more resistant to infection upon curing following digging.

More PMA was deposited on the bulb when unformulated PMA was used than when a formulated preparation containing the same amount of PMA was used. The percentage of rotted bulbs decreased as the amount of deposited PMA increased, but this decrease was not in direct proportion to the PMA deposited. PMA formulated with thiomalic acid at pH 10.5 appeared to prevent rot as effectively as did unformulated PMA when used in concentrations that left an equivalent amount of fungicide on the bulb; however, considerably higher concentrations of formulated PMA were required in the dip.

DISCUSSION.—Formulations of PMA available in the United States do not appear to prevent adsorption by soil; however, an ethylmercury formulation obtained from Holland (AA Bulba) was tested by the procedure reported here and found to be stable in the

TABLE 6.—*Amount of phenylmercury acetate (PMA) deposited on narcissus bulbs and percentage of bulbs rotted, following inoculation of Golden Harvest bulbs with Fusarium oxysporum f. narcissi and subsequent treatment with formulated and unformulated dips containing various concentrations of PMA*

Formulation			PMA on bulb (ppm)		Percentage of bulbs rotted	
PMA (ppm)	Stabilizer[a]	pH	Trial 1[b]	Trial 2[c]	Trial 1[b]	Trial 2[c]
None			0.0	0.0	94	39
18	None	5.0	3.5		55	
200	None	5.0	20.2	17.1	19	11
200	Thiomalic acid + Na_2CO_3	10.5	4.2		30	
1400	Thiomalic acid + Na_2CO_3	10.5		27.1		7
2200	Thiomalic acid + Na_2CO_3	10.5	36.6		11	

[a] Stabilizer added according to the equation:
$RHgOAc + R'SH \rightarrow RHgSR' + HOAc$.
[b] Trial involved bulbs freshly dug and of high quality, 500 per treatment.
[c] Trial involved bulbs of poorer quality, 400 per treatment.

presence of soil. Also, the active ingredient of a fungicide available in Germany known as Fusariol is reported to be ethylmercury cyanide (2), which would be stable to soil. Ceresan 200, an ethylmercury formulation available in the United States, is partially stabilized. If this type of formulation becomes generally available, field practices must be adjusted for their use.

In the usual bulb-treating procedure, loss of PMA occurs from the treating solution. Compensation for this loss may be made by addition of PMA at regular intervals to the treating tank or by formulation to prevent loss from contact with iron, wood, or soil.

Tests for the decomposition of PMA via diphenylmercury were inconclusive. Although the alkaline thiol formulations promoted this decomposition, subsequent laboratory tests failed to show lessened fungicidal activity from these formulations. It is interesting to note that the relatively stable thiomalic acid caused much less diphenylmercury to form in alkaline solution than did the less stable thioglycollic acid. The addition of sodium sulfite to prevent oxidative decomposition of the thioglycollic acid was of no value. Thioglycollic acid formulations of PMA in weakly acid solution (pH 4–6) are not stable for long periods of time (several months), whereas thiomalic acid formulations appear to be stable indefinitely in the 200-ppm range, especially at pH 10.5.

The data from the tests with inoculated bulbs (Table 6) do not indicate that the formulations decrease the fungicidal potency of PMA; however, the amount of phenylmercury deposited on the bulb from unformulated PMA is much greater than from formulated. Therefore, the control of basal rot may be dependent on the amount of phenylmercury remaining on the bulb. The flowers from bulbs from the treatments in Table 6 showed no evidence of mercury injury (9), even those from bulbs dipped in the 2200-ppm formulated PMA.

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PHYSIOLOGICAL STUDIES OF DOTHIDELLA ULEI [1]

Carlos H. Blazquez and John H. Owen[2]

SUMMARY

The foliage of rubber trees, *Hevea brasiliensis* (Muell.) Arg., growing in a greenhouse, was inoculated with strains of *Dothidella ulei* P. Henn. Severe outbreaks of disease occurred from May through November whenever sufficient rain or foggy weather increased the atmospheric moisture to high levels.

Growth of the fungus *in vitro* was obtained on media containing leaf extract of *H. brasiliensis*, or extracts of certain other plants, on semisynthetic media, and on semisynthetic media containing combinations of 5 vitamins and 5 amino acids. The media that gave the best growth were one containing extract from 20 g of Hevea leaves and 2.5 g of malt extract per liter and one consisting of Difco lima-bean agar (23 g/1 containing quebrachitol, 200 mg/1). The most favorable combinations of a vitamin and an amino acid in media were *i*-inositol plus glutamine and nicotinic acid plus glycine; on these, colonies ranged from 5 to 16 mm in diameter after 26 days of incubation. Conidial production was most abundant on combinations of riboflavin with glutamine, leucine, arginine, or glycine. On such media, the fungus first produced a small olivaceous cluster of mycelium and then gradually formed a blackish mass of stromatic tissue that was either submerged or raised above the surface. Spermogonia were plentiful on the raised type of growth, and conidia were developed on the surface of the stromatic tissue; the submerged growth formed some olivaceous mycelium and conidia on the surface of the medium.

Spore germination occurred best on media at pH 7 and 8 and at temperatures of 24°–28°C.

Growing the fungus *in vitro* did not appear to decrease its pathogenicity.

Dothidella ulei P. Henn. causes a destructive foliage disease of the Para rubber tree, *Hevea brasiliensis* (Muell.) Arg., that is known as the South American leaf blight disease. It has been the primary cause of the failure of many plantations in the Guianas and of the Ford plantations at Fordlandia, Belterra, and at Belem, Brazil.

The fungus sporulates abundantly on the infected host tissue during the rainy season in those areas where it is endemic, and spores suitable for inoculation purposes are usually abundant during these wet periods. It is impossible to test the resistance of new rubber tree progenies in those areas where the disease does not occur, since the fungus has not been grown on artificial culture media. The fungus has been considered to be an obligate parasite by many workers (2, 3, 8). Kuyper (3), in 1911, reported that the conidia would germinate on a variety of media but would not grow on any of them. He concluded that the ability of the fungus to grow on young Hevea leaves and not on artificial media might be due to the presence of large amounts of hydrogen cyanide in the former and the difficulty of providing the right concentration of this material in the medium. He also tried decoction medium from *Hevea* spp. but obtained no growth of the fungus. Stahel (9), in 1917, made extensive studies of the organism and also germinated the conidia on a large number of media but was unable to culture the fungus. Langford (4), in 1945, could not culture the fungus on ordinary nutrient media such as potato-dextrose agar; however, he was successful in obtaining slow-growing colonies on malt agar containing extract from leaves of *Hevea* spp.

The purpose of this research was to attempt to obtain growth *in vitro* of *D. ulei* and to determine if possible the factor or factors that influence the growth of this fungus.

MATERIALS AND METHODS.—Seedlings of *Hevea brasiliensis* from a cross of clones Tjirandji 1 × Tjirandji 16 were obtained from the U. S. Plant Introduction Gardens at Coconut Grove, Florida. This cross, of Liberian origin, is high yielding and is highly susceptible to the leaf blight disease. The seedlings were grown in 8-in. pots in a greenhouse maintained as near as possible at the optimum temperature for the growth of the seedlings (25°–27°C). The relative humidity was maintained at as high a level as was possible in the greenhouse. Moisture was provided by a mist-type sprayer. The fastest growing trees were topped to keep them at a workable height and to produce more young, susceptible foliage.

Leaves of *H. brasiliensis* infected with *D. ulei* were obtained from Panama, Mexico, Costa Rica, and Guatemala, and the conidia from these leaves were used to inoculate the foliage of the Hevea seedlings.

Once initial infection had been obtained, periodic outbreaks of the disease occurred on young foliage from May through November, the main outbreaks being correlated with periods of rainfall. Between rainfalls, the atmosphere inside the greenhouse was extremely dry, and no new infection occurred unless inoculated foliage was kept under continuous mist. This infected foliage provided a continuous source of conidia for use in studies of the fungus *in vitro*.

HOST INOCULATION.—A wet camel's-hair brush was used for picking up the conidia from the diseased leaf

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and for brushing them onto the moistened under surface of the young leaves. The inoculated parts were covered with plastic bags unless the humidity in the greenhouse was high.

In the determination of the period of susceptibility of the foliage, trees with leaves of various ages were inoculated under extremely favorable conditions of humidity and temperature and observed daily for a period of 25 days. Leaves were considered to be 1-day-old when they were ¾ in. in length. The leaves were first sprayed with a mist sprayer and then inoculated. Most of the inoculated leaves from 2 to 12 days old were showing symptoms within 2–3 days or, in some cases, within 36 hours after inoculation. In the very young stages, the leaves and petioles were usually killed within a week and were shed. The very young infected tissues turned black and did not develop conidia until almost ready to fall. Conidial production was most abundant on infected leaves 2–10 days old, and typical symptoms developed on these leaves (Fig. 1, E). Some of the older inoculated leaves were killed immediately because of an excessive amount of inoculum, but many leaves with few lesions remained on the trees. Inoculation of leaves older than 20 days resulted in no infection, although slight infection did occur on the 21-day-old leaves of 1 tree.

Growth of the fungus in vitro.—It was first necessary to find a medium suitable for spore germination. Since Langford (4) had obtained some growth on a medium containing leaf extract and malt extract, this was the first type of medium tested for spore germination. A high percentage of germination occurred on a medium containing extract from 20 g Hevea leaves plus 2.5 g malt extract per liter. The conidia were brushed from leaf lesions by means of a camel's-hair brush onto petri dishes of this medium. The seeded plates were incubated at 20°–24°C for 30 hours and the conidia allowed to germinate and develop some growth before they were transferred to slants of media to be tested. Hard agar media were chosen for these studies, and agar slants in test tubes were used instead of petri dishes to minimize contamination. All cultures were incubated at 20°–24°C.

Surface-sterilized leaf lesions.—Young 4-day-old lesions, which were in the light green stage, were surface sterilized for 1 minute in a 1:2000 solution of mercuric chloride, rinsed twice in sterile distilled water, dissected with a flamed scalpel, and placed on potato-dextrose agar and also on a medium containing an extract from 50 g of Hevea leaves plus 5 g of malt extract per liter. Although the fungus grew and developed stromatic tissues and spores on the host tissues, it did not grow out onto the surface of either media.

Media containing leaf extract of Hevea brasiliensis.—Fifty g of young Hevea leaves were cut into small squares and boiled in distilled water for 30 minutes. Five g of malt extract and 20 g of agar were dissolved in the decoction and the contents made up to 1 liter with distilled water. No spore germination was obtained in this medium. A second leaf-decoction medium contained extract from 25 g of young leaves and 5 g of malt extract. The fungus grew slowly on the surface of this medium and consisted of small, dark, superficial masses of stromatic tissue, with spermogonia developing throughout the stroma after 5 weeks.

An experiment was set up to determine the most favorable concentrations of leaf extract and malt extract for the growth of the fungus. Extract from 25 g of leaves and 5 g of malt extract were mixed with water agar in proportions that reduced the amount of leaf extract from one equivalent to 25 g to one equivalent to 2.5 g per liter, at intervals of 2.5 g, with the malt extract being reduced proportionately. The best growth was obtained with medium containing extract from 15 g of leaf tissues and 2.5 g of malt extract per liter. Conidia seeded on these media germinated readily and grew slowly; the resultant colonies formed stromatic tissue on the surface of the medium, but no conidia were produced. One week after the seeding, conidia were transferred to potato-dextrose-agar slants and incubated at 24°C. After the transfer, these colonies had grown to a diameter of 1 mm within 1 week and to one of 6 mm after 1 month; after 5½ months, the colonies had covered the surface of the slant. Some mycelial formation was noted on the medium, and spermogonia developed in the stromatic tissue, producing an abundance of spermatia. Growth, however, was mostly on the surface of the medium, and there were no visible signs of submerged mycelium (Fig. 1, F). The height of some stroma above the surface of the medium was 6–7 mm. Spermogonia were observed after 2 weeks or as soon as the stromatic tissue had become black and carbonaceous in appearance. They were imbedded in the stromatic tissue, with only the ostioles protruding. The ostioles of the spermogonia were visible after the fifth week; after 5 months, spermatia were observed exuding from the ostioles in glossy droplets. Paraffin sections

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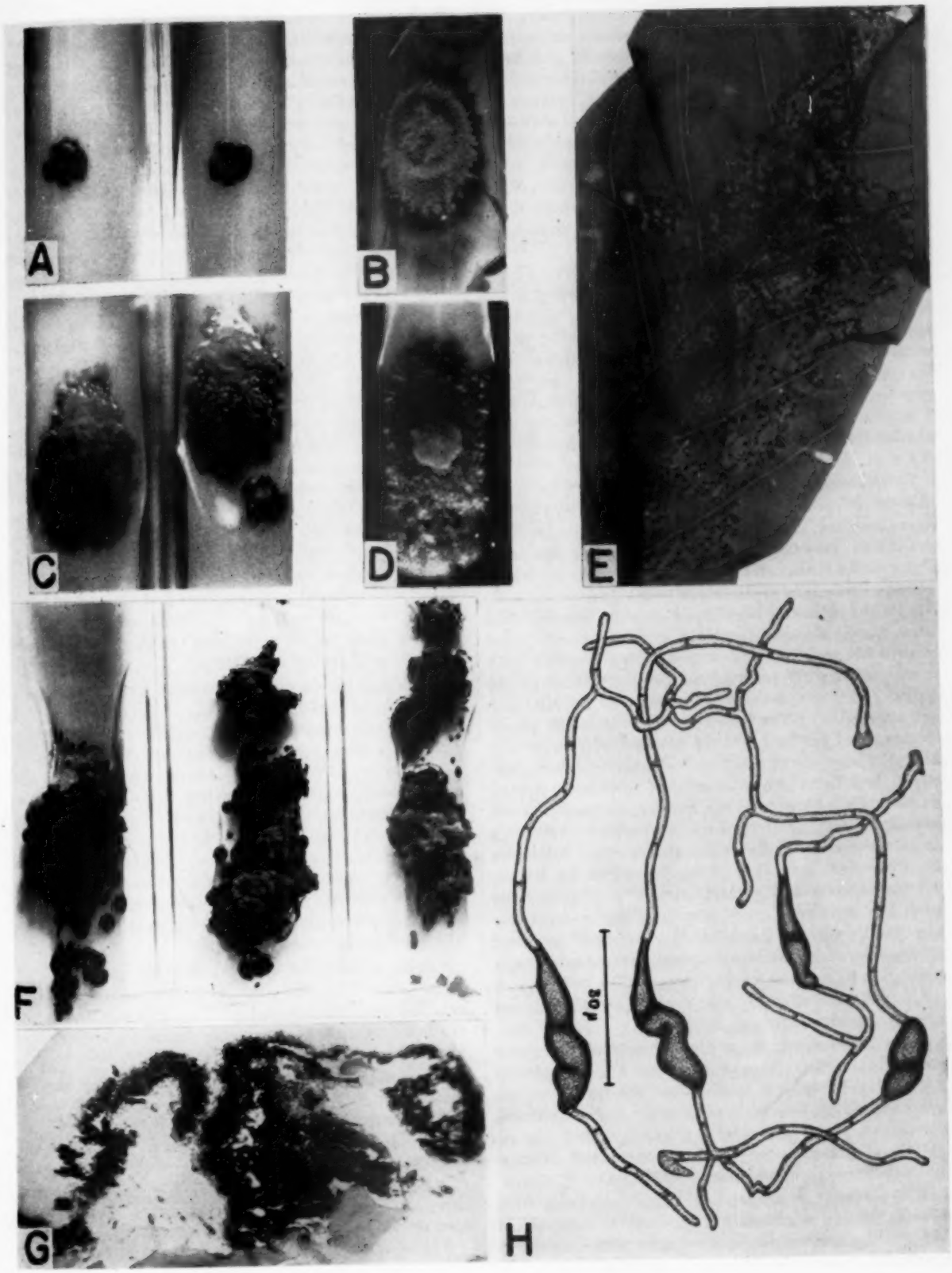
Fig. 1. *Dothidella ulei.* A) Forty-day-old cultures on agar medium containing malt extract (2.5 g/l) and extract of Hevea leaves (20 g/l). B) A 100-day-old culture on solid semisynthetic medium containing *i*-inositol and glutamine. C) A 90-day-old culture on Hevea leaf extract (20 g/l) and malt extract (2.5 g/l). D) A 100-day-old culture on agar medium containing nicotinic acid and glycine. E) Severely infected leaflet showing an abundance of conidia. F) A 165-day-old culture seeded on agar medium containing Hevea-leaf extract (15 g/l) and malt extract (2.5 g/l) and transferred to potato-dextrose agar. G) Paraffin section of culture shown in F. H) Camera lucida drawing of germinating conidia.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of these colonies revealed that they were hollow and not solid masses of stromatic tissue (Fig. 1, G).

A modification of the leaf-decoction, malt-extract medium was prepared with extract from 20 g of leaves and 2.5 g of malt extract per liter. Growth on this medium differed from that observed on potato-dextrose agar; stromatic masses of the fungus were submerged in the medium, forming only conidiophores and conidia on the surface. In some cultures, white mycelium developed along the periphery of the colony. No spermogonia were observed. The average diameter of the colonies was 5.5 mm after 40 days (Fig. 1, A) and 18 mm in 100 days (Fig. 1, C).

Media containing other plant extracts.—The fungus did not grow on Difco malt-extract agar (33.6 g per liter). On Difco malt-extract agar (33.6 g per liter) supplemented with 1 g/l of quebrachitol (2 monomethyl ether of *i*-inositol, which occurs in Hevea latex in concentrations of 0.5 to 2 per cent), only slow-growing colonies developed; these were not more than 1 mm in diameter after 53 days. Similar results were obtained when this medium was supplemented with 6.5 g of Hevea leaves per liter.

Difco lima-bean extract agar appeared to be favorable for growth. On medium containing 23 g/l of this agar, both the spermogonial and conidial stages were produced; growth was very slow, however, for after 5½ months colony diameter was only 8 mm. Where 11.5 g of this agar medium per liter was used, colonies 8 mm in diameter developed in 5½ months and both spermogonia and conidia were produced. Good growth occurred on Difco lima-bean agar (23 g/l) containing 1 g of quebrachitol per liter, although the colonies did not develop as rapidly as on the leaf-extract media. Difco lima-bean agar with 200 mg of quebrachitol per liter was the best medium for growth except those containing leaf extract. Growth was rapid, and the colonies developed olivaceous hyphae and conidia within 2 weeks. Spermogonia were formed within 6 weeks, and spermatia were observed exuding from the ostiole of the spermogonia after 2 months. On Difco lima-bean agar (9.2 g/l) containing 400 mg of quebrachitol and extract from 5 g of leaves per liter, the mycelium grew deep into the medium and also on the surface (dark olive); the latter produced an abundance of conidia but no spermogonia. Colonies grew to a diameter of 5 mm in 2½ months.

On V-8 juice agar (7), the conidia germinated very rapidly but developed no further.

Growth was scanty on medium containing 2 per cent polished-rice extract; colonies reached a diameter of 2 mm after 6 weeks of incubation. The mycelium was generally olivaceous, with some white hyphae forming along the periphery of the colonies. The colonies developed primarily on the surface of the medium and produced an abundance of conidia.

Water agar (20 g agar per liter) containing from 100 mg to 1 g quebrachitol per liter at intervals of 100 mg was prepared. No growth occurred on media containing more than 800 mg/l. Best growth was at concentrations of 100 mg/l. Young colonies, plus a small portion of medium approximately ½ mm^3 in size, were transferred from the media containing 100, 200, 400, and 600 mg quebrachitol per liter to slants of medium containing extract from 20 g of leaves plus 2.5 g of malt extract per liter. Incubation was at 20°C. All cultures grew rapidly, producing colonies that averaged 5 mm in diameter in 10 days. In 90 days, the average diameters of the cultures were 15, 11, 12, and 16 mm, respectively. After 140 days, they measured 23, 16, 20, and 21 mm, respectively. The fungus produced shiny black submerged mycelium that grew to a depth of 2 mm and came to the surface of the medium at various intervals, producing an abundance of conidia.

Semisynthetic media.—A basal semisynthetic medium was prepared as follows: dextrose, 10 g; asparagine, 2 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $(Fe_2(SO_4)_3 \cdot XH_2O)$, 0.2 mg; $(ZnSO_4 \cdot 7H_2O)$, 0.2 mg; $(MnSO_4 \cdot H_2O)$, 0.1 mg; biotin, 5 μg; thiamine, 100 μg; agar, 20 g; and distilled water to make 1 liter. Growth on this medium was extremely slow. The conidia germinated well and formed olivaceous colonies about 1 mm in diameter; however, no further development occurred. Growth was good on basal semisynthetic medium containing extract from 8.3 g of leaves and 1.16 g of quebrachitol per liter. A black, raised stromatic tissue formed on the surface of the medium, and conidiophores and white mycelium developed on the surface of this tissue. The fungus did not grow on semisynthetic medium plus extract from 25 g of leaves per liter.

Semisynthetic medium containing various amounts of quebrachitol (up to 1 g/l) was prepared. No growth was obtained on media containing more than 400 mg of quebrachitol, but some growth occurred at all lower concentrations. Growth was best at the 100-mg level, the lowest level tested. On this medium, colonies measured 5 mm in diameter after 2 months and consisted only of stromatic tissue with no olivaceous mycelium or spermogonia. Olivaceous mycelium and an abundance of conidia were produced on Czapek's sucrose-nitrate agar (6). Growth was on the surface of the medium, and after 6 weeks the colonies had attained a diameter of 7 mm.

Effects of vitamins and amino acids on growth of fungus.—Five vitamins and 5 amino acids in various combinations were added to lots of the basal semisynthetic medium as previously described, except that 42 mg/l of asparagine was used.

Conidia of the fungus were seeded on the various media. After 2 days, a mass of germinated conidia and a small section of the medium from each culture were transferred from each to slants of the same kind of medium. Observations of the growth and progress of these cultures were made during 4 months' incubation at 20°–24°C; results were recorded after 35 days and 100 days.

The medium on which the best growth and conidial production occurred was *i*-inositol plus glutamine

(Table 1). Although the average diameter of the colonies was 3 mm after 35 days and 7 mm after 100 days, 1 culture had attained a diameter of 16 mm in 35 days and one of 30 mm in 100 days (Fig. 1, B). The fungus in this slant produced white and olivaceous mycelium that grew into the medium to a depth of 3–6 mm. About 50 per cent of the surface mycelium was sporulating after 35 days, and the formation of conidia increased as the culture aged, producing an abundance of conidia after 100 days of growth.

The fungus did not grow on media containing *i*-inositol plus arginine or on that containing *i*-inositol alone.

The fungus produced a raised, black, erumpent stromatic tissue on the surface of the media supplemented with pyridoxine only or with *p*-aminobenzoic acid alone; conidial and spermogonial production were plentiful on these media.

TABLE 1.—*Growth, spermogonial production, and conidial production by Dothidella ulei on a semisynthetic medium containing different combinations of various vitamins and amino acids*

Vitamin and amino acid[a]	Spermogonial formation[b]		Conidial production[b]		Average diameter of colony (mm)	
	35 Days	100 Days	35 Days	100 Days	35 Days	100 Days
i-Inositol (400 mg)						
Glutamine (21 mg)	1	1	2	4	3[c]	7
Aspartic acid (21 mg)	0	2	0	2	0	2
Leucine (21 mg)	1	1	0	0	1	6
Arginine (63 mg)	0	0	0	0	0	0
Glycine (21 mg)	0	0	3	3	3	8
None	0	0	0	0	0	0
Pyridoxine (5 mg)						
Glutamine (21 mg)	3	3	2	2	5	5
Aspartic acid (21 mg)	0	0	0	0	0	0
Leucine (21 mg)	0	0	0	0	0	0
Arginine (63 mg)	1	1	1	2	1	4
Glycine (21 mg)	3	3	2	3	5	5
None	2	3	2	3	4	6
p-Aminobenzoic acid (5 mg)						
Glutamine (21 mg)	1	2	1	1	1	3
Aspartic acid (21 mg)	1	3	1	2	1	5
Leucine (21 mg)	1	1	0	0	1	3
Arginine (63 mg)	0	3	1	1	2	5
Glycine (21 mg)	2	2	1	2	1	4
None	1	3	0	3	0.25	7
Nicotinic acid (2 mg)						
Glutamine (21 mg)	0	0	0	2	0	7
Aspartic acid (21 mg)	0	0	0	0	0	0
Leucine (21 mg)	0	0	0	2	1	4
Arginine (63 mg)	0	0	0	1	0	2
Glycine (21 mg)	0	2	3	3	4	7
None	0	0	0	2	0	3
Riboflavin (2 mg)						
Glutamine (21 mg)	3	3	2	2	5	7
Aspartic acid (21 mg)	0	0	0	0	0	0
Leucine (21 mg)	1	1	2	3	3	6
Arginine (63 mg)	2	2	2	2	3	3
Glycine (21 mg)	0	1	2	3	3	4
None	0	0	0	0	0	0

[a] Figures in parenthesis are amounts used per liter.

[b] Arbitrary numerical rating for percentage of surface mycelium that produced spermogonia and conidia as follows: 4, above 75%; 3, 50–75%; 2, 25–50%; 1, below 25%; 0, none.

[c] One colony reached a diameter of 16 mm in 30 days and 35 mm in 100.

At first, growth was slow with medium containing nicotinic acid plus glycine, but after 35 days the fungus had formed black mycelium that penetrated deep into the medium. Olivaceous mycelium developed on the surface of the medium and produced an abundance of conidia and spermogonia (Fig. 1, D).

On medium containing riboflavin plus glutamine, the fungus formed submerged black stromatic tissue deep in the medium and on the surface of the medium, where it obtained a height of 3 mm. Olive-colored mycelium was observed on the surface of the stromatic tissue, and conidial formation and spermogonia were plentiful.

On a medium containing riboflavin plus glycine, growth was fair and 1 culture grew to a diameter of 8 mm and produced submerged black mycelium and raised olivaceous tufts of conidiophores. Sporulation by this culture was abundant, and the yellow color (riboflavin) disappeared as the fungus growth increased.

Spermogonial formation in cultures appeared to be suppressed somewhat by the presence of nicotinic acid and of *i*-inositol.

INOCULATION OF HOST WITH FUNGUS GROWN IN VITRO.—Spore suspensions were prepared with conidia from three 2-month-old cultures grown on medium containing leaf extract and malt extract. The foliage of 3 trees with 6- to 7-day-old leaves was inoculated by the brush method. Favorable weather developed 2 days after inoculation, and lesions were visible within 5 days after inoculation. The fungus infected foliage on all of the inoculated trees, and no differences were noticed between lesions produced by the conidia taken from infected leaves and those produced by conidia grown in culture. Pathogenicity of the fungus did not appear to be affected by growth *in vitro*. Conidia developing on these lesions were sown and transferred onto leaf extract, malt extract medium; colonies consisting of olivaceous mycelium grew deep into the medium, and after 3 weeks had reached a diameter of 4 mm.

SPORE GERMINATION.—The effects of pH and temperature on germination of conidia were determined.

Effect of pH.—Buffer solutions ranging from pH 3 to pH 9, at intervals of 1 pH unit, were prepared with Hydrion Buffer Standards (Micro Essential Laboratory, Brooklyn, N. Y.) in distilled water. Conidia were brushed from the lower surfaces of leaf lesions into 3 ml of each solution, and these were incubated at 22°C. Forty per cent of the conidia at pH 7 and at pH 8 had germinated within 3 hours, and 20 per cent of the conidia at pH 5 and pH 6 had germinated within 9 hours. Germination was best at the higher pH values, although conidia germinated at all pH values tested. This differs from the results obtained by Stahel (9), who found that germination was good only in acid media.

Temperature.—Conidia were seeded onto petri dishes of agar medium containing extract from 20 g of leaves plus 2.5 g of malt extract per liter, and 2 dishes were incubated at 4°, 8°, 12°, 16°, 20°, 24°, 26°, 28°, and 32°. The best germination occurred at 24°, 26°, and 28°C. Germ-tube development was similar at 20° and 32° for the first 6 hours; after 9 hours, conidia at 20° were 200 μ or more in length and those at 32° were 100 μ in length. Germ-tube development at 16° was rather slow during the first 12 hours but increased rapidly from 12 to 24 hours. Germ-tube development at 8° and 4° was similar to that at 16°; germ tubes appeared within 2–3 hours and developed at a very slow rate, not attaining more than 30 μ in length over a 48-hour period. The conidia were usually 2 celled, with the terminal cell being slightly twisted and the larger. At 4° and 8°, the larger cell of the conidium germinated first, with the second cell producing a germ tube when the one formed by the larger cell was about 5 μ in length. The germ tubes developed unequally for about 3 hours, after which time the germ tube of the smaller cell began to enlarge at a rapid rate; it eventually became almost as long as the germ tube from the larger cell (Fig. 1, H). Germ-tube development at 12° was considerably retarded; germ tubes attained a length of about 140 μ during a 48-hour period.

The germ tubes first grew upward almost perpendicular to the surface of the medium, with the conidium being the only structure in contact with the surface of the medium. At the higher temperatures, frequent branching was common; at 28°, profuse branching was observed. At 32°, the germ tubes appeared to be growing very slowly, had a stunted appearance, and branched close to the originating cells. In some instances, germ tubes came in contact with the medium and apparently ceased to develop any further.

Discussion.—The failure of preliminary attempts to germinate conidia and grow the fungus on artificial culture media was due primarily to the lack of a favorable system of seeding the conidia on the media and to high concentrations of leaf extract in the media. The first attempts to germinate the conidia gave results similar to those obtained by Kuyper (3) and Stahel (9), as conidia either did not germinate or germinated readily but ceased growing after 48 hours. Spore germination is affected not only by nutrition but also by viability and age of the conidia, by type of medium, by pH, and by the temperature during incubation. When the conidia were first germinated on a favorable medium and allowed to form small colonies, colonies transferred to a wide variety of media would continue to grow.

The toxicity of high concentrations of *i*-inositol to *D. ulei* is in agreement with the findings of Barnett and Lilly (1) that *i*-inositol has a toxic effect on the growth of *Sclerotinia camelliae* Hara at high temperatures and high concentrations. There may be some relationship between the toxic effects of high concentrations of both quebrachitol and *i*-inositol, as both compounds are very similar in structure. Quebrachitol is present in the latex of species of *Hevea* in concentrations of 0.5 to 2.0 per cent; however, it may be present in smaller amounts in the latex of young leaves. This may be the reason why young Hevea leaves are susceptible to *D. ulei* whereas older leaves are not or only slightly so.

The fungus produced either submerged or raised growth on the various media, indicating that such media are either favorable or slightly antagonistic to the fungus. Apparently, the small transfer block of medium containing Hevea-leaf extract and malt extract contained enough nutrients for the fungus colony to continue growth and gradually to become adapted to potato-dextrose agar, since it would not grow on this medium alone.

The diameter of the colonies was not an accurate measure of fungus growth, as some colonies were submerged in the medium to a depth of 8 mm, whereas others grew to a height of 8 mm above the surface of the medium.

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PATHOGENIC, CULTURAL, AND PHYSIOLOGICAL VARIATION WITHIN PHYTOPHTHORA PARASITICA VAR. NICOTIANAE[1]

J. L. Apple[2]

SUMMARY

Over 200 isolates of the black shank fungus were collected from 55 North Carolina counties, from 9 other states, and from 4 foreign countries. Most of these isolates were compared in inoculation tests and in culture.

By use of a greenhouse seedling inoculation technique, it was found that isolates of the black shank fungus varied from a low level of pathogenicity on a susceptible tobacco variety to a high level of pathogenicity on resistant varieties.

The increase of these highly pathogenic biotypes in fields planted continuously to a resistant variety can probably take place through adaptive selection. It is likely that the concentration of highly pathogenic biotypes within a given field is a factor contributing to the current black shank disease problem in resistant varieties. This is supported by the fact that isolations from severely diseased crops of a resistant variety yielded a higher percentage of highly pathogenic biotypes than did isolations from similar crops of a susceptible variety.

Isolates obtained from single zoospores from the same thallus exhibited pathogenic and cultural variations comparable to those of natural populations.

In cultural morphology and in response to hydrogen-ion concentration, the black shank fungus present in the burley-producing areas of Kentucky, Tennessee, and western North Carolina differs significantly and consistently from that present in the flue-cured-tobacco area of North Carolina and adjoining states. These differences are considered adequate to justify designating the 2 types as distinct cultural strains of *Phytophthora parasitica* var. *nicotianae*, viz. the burley strain and the flue-cured-tobacco strain.

Phytophthora parasitica (Dast.) var. *nicotianae* (B. de Haan) Tucker, the causal organism of the black shank disease of tobacco, is widespread throughout the tobacco-growing areas of southeastern United States. The production of tobacco in these infested areas is possible only through the use of resistant varieties.

The first flue-cured tobacco varieties resistant to black shank were released in 1943 (5), with additional varieties being released in 1949 (8) and 1955.[3] All of these varieties have a common source of black shank resistance that is complex in inheritance. The levels of resistance range from moderate to high, the latter being comparable to that of the resistant parent, Florida 301, a cigar-wrapper type (15). In North Carolina, resistant varieties generally gave satisfactory disease control through the 1950 growing season; however, their performance during the 1951 season was erratic. Many fields were observed in which 50–95 per cent of the plants of moderately resistant varieties had been killed by the black shank organism. The number of tobacco fields in which unusually heavy losses occurred in resistant varieties increased progressively in 1952, 1953, and 1954. Many factors, such as root injury induced by plant parasitic nematodes (11), unfavorable environmental conditions, and inoculum potential, may have contributed to the unexpected failure of these varieties in some fields; however, this paper is concerned only with the possibility of the existence of pathogenic strains within *P. parasitica* var. *nicotianae*.

No previous comprehensive study has been made of the black shank fungus with respect to variability. Nolla (9) reported a weakly pathogenic strain obtained from Sumatra but concluded that this was probably due to the old age of the culture. A weakly pathogenic strain from North Carolina was reported by Powers and Lucas (10).

Since the variation potential of the black shank fungus has not been considered in breeding for disease resistance, the present study was initiated to determine 1) the frequency of occurrence and pathogenic range of variants in this organism, 2) whether such pathogenic variation is related to the present black shank disease problem in resistant varieties, and 3) whether certain morphological or physiological characteristics of various isolates are related to pathogenicity and/or source. A preliminary report has been published (1).

MATERIALS AND METHODS.—The 225 isolates of *P. parasitica* var. *nicotianae* acquired during this study were obtained as follows: 184 from 55 counties in North Carolina, 1 from California, 10 from Florida, 4 from Georgia, 8 from Kentucky, 1 from Maryland, 1 from Pennsylvania, 6 from Tennessee, 4 from Virginia, 1 from South Carolina, 2 from Java, 1 from Sumatra, 1 from India, and 1 from Puerto Rico. The pathogenicity of most of these isolates was determined

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[3] Varieties Coker 139 and Coker 140 released by the Coker Pedigree Seed Company, Hartsville, South Carolina.

in greenhouse inoculation studies.

Initial pathogenicity determinations were made with 4 replicates, 2 of a susceptible and 2 of a resistant variety. Subsequent evaluations were made with 8 replicates (72 plants), 4 of the susceptible Bottom Special and 2 each of the resistant varieties Coker 139 and Dixie Bright 101. Plants were grown in 8 × 8 × 2-in. aluminum trays of sandy loam soil treated with methyl bromide. Nine seedlings were grown in each tray. Inoculations were made when the plants were in the 4- to 5-leaf stage by flooding with 100 ml of a mycelial suspension prepared by blending the contents of a 2-week-old oatmeal-agar plate with 200 ml of distilled water in a Waring Blendor. Disease symptoms appeared on the second or third day after inoculation. Daily counts of obviously diseased and dying plants were made during the 12-day period following inoculation and used for the computation of a pathogenicity index for each isolate. Diseased plants were placed in a class that corresponded to the number of days required for the expression of symptoms after inoculation. The classes and corresponding class values were as follows: 3 days—10; 4 days—9; 5 days—8; 6 days—7; 7 days—6; 8 days—5; 9 days—4; 10 days—3; 11 days—2; and 12 days—1. In the calculation of the pathogenicity index for an isolate, the number of plants placed in each class was multiplied by the corresponding numerical value; these products were totaled and the totals divided by 9, the number of plants in each tray. Thus, the pathogenicity indices reflect both the extent and the rapidity of disease development. In order to check the validity of the isolate characterizations based on seedling inoculations, selected isolates were used to inoculate larger plants growing in 6-in. pots.

Oatmeal agar was used for maintaining stock cultures and for producing inoculum for greenhouse studies. Fifty g of Quaker Oats were soaked in distilled water for several hours, then blended in a Waring Blendor. Fifteen g of agar was added and then enough distilled water to bring the volume up to 1000 ml. When prepared in this manner, the medium was of uniform consistency and always gave excellent growth.

Stock cultures were stored at 15°–20°C. It was found that storage at lower temperatures greatly reduced the viability period of the cultures.

The medium used in pH studies was prepared from fresh tobacco leaf extract. One hundred g of leaf tissue per 200 ml of distilled water were ground in a Waring Blendor, and the resulting material was forcibly strained through cheesecloth. The extract thus obtained was filtered twice under suction through medium-grade filter paper. One liter of medium contained 200 ml of the filtrate, 10 g of dextrose, and 18 g of agar. After sterilization, this was buffered by the addition to each 100 ml of medium of 5 ml of the following mixture: citric acid, 10.6 g; glycine, 3.8 g; KH_2PO_4, 4.6 g; and distilled water, 100 ml. Desired pH levels were obtained by the addition of NaOH or HCl solution. The data were taken 6 days after the plates were seeded with the fungi. Colony diameters were measured in mm; the data were converted to log (colony diameter + 1) before being subjected to a multiple regression analysis with a second-degree polynomial in pH fitted to the responses (growth).

In comparisons of the cultural morphology of various isolates, tobacco-extract agar and 20 per cent V-8 juice (a mixture of vegetable juices manufactured by Campbell Soup Co.) agar (6) were used.

EXPERIMENTAL RESULTS.—*Pathogenic variation.*—Initial inoculation studies revealed extreme pathogenic variation within *P. parasitica* var. *nicotianae*, as demonstrated by the pathogenicity indices in Table 1. Isolates varied from nonpathogenic on the susceptible variety to highly pathogenic on the resistant varieties used. Isolate 309, intermediate in pathogenicity, and isolate 906, weakly pathogenic, were included in each of 45 inoculation tests as controls, and they maintained this relationship to each other in every test.

Based on the performance of isolate 309, 3 arbitrary isolate classes were established as follows: isolates with a significantly higher pathogenicity index (at 5 per cent level) than the 309 check were classed as highly pathogenic, those significantly lower were classed as weakly pathogenic, and all others were

TABLE 1.—*Pathogenicity indices of representative inoculation test showing pathogenic variability within Phytophthora parasitica var. nicotianae*

Isolate	Test variety[a]		Isolate Mean
	Bottom Special	Dixie Bright 101	
	(Highly pathogenic isolates)		
319	9.4	2.5	6.0
320	9.4	2.4	5.9
322	9.8	2.4	6.1
340	7.5	3.3	5.4
378	9.2	3.2	6.2
398	8.2	2.5	5.4
423	7.8	4.0	5.9
	(Isolates intermediate in pathogenicity)		
229	7.9	0.6	4.2
302	4.8	0.3	2.6
318	7.2	2.2	4.6
336	4.1	0.9	2.5
393	6.0	0.7	3.4
309	6.6	0.9	3.8
	(Weakly pathogenic isolates)		
339	2.6	0.0	1.3
354	4.2	0.0	2.1
358	1.6	0.0	0.8
906	3.7	0.0	1.9

[a] Bottom Special—susceptible; Dixie Bright 101—moderately resistant. Two replicates were used on each test variety. Daily counts of diseased plants were made during the 12-day period following inoculation. Diseased plants were placed in a class that corresponded to the number of days required after inoculation for the expression of symptoms. The classes and class values were as follows: 3 days–10; 4 days–9; 5 days–8; 6 days–7; 7 days–6; 8 days–5; 9 days–4; 10 days–3; 11 days–2; 12 days–1. LSD between isolate means: 5 per cent = 1.6; 1 per cent = 2.2; LSD between isolate means on the same variety: 5 per cent = 1.5; 1 per cent = 2.0.

classed as intermediate. On this basis, 133 isolates from North Carolina were grouped as follows: 53 highly pathogenic, 66 intermediate, and 14 weakly pathogenic. Of these 133 isolates, 57 were isolated from resistant varieties and 76 from susceptible or unknown varieties. Of those from resistant varieties, 51 per cent were highly pathogenic; in contrast, only 32 per cent of those from susceptible or unknown varieties were highly pathogenic. These data suggest a correlation between level of pathogenicity and the history of the isolate.

The consistency of the inoculation procedure was demonstrated when 12 isolates were used in 2 successive experiments. Data from both experiments were included in a split-plot analysis. The experiment × isolate interaction was significant (5 per cent level) with only 2 isolates. All others performed consistently.

The validity of isolate characterizations made on the basis of seedling inoculations was checked by inoculating larger plants. The plants were grown in 4-in. pots until about 12 in. tall. They were inoculated when transplanted into 6-in. pots by dipping the ball of soil and roots into a mycelial suspension. The results (Table 2) showed good agreement with those obtained in seedling inoculation experiments. Plants inoculated in this manner with isolates that varied in pathogenicity from low to the highest level found demonstrated clearly that some pathogenic strains of *P. parasitica* var. *nicotianae* are capable of killing plants of resistant tobacco varieties currently in use (Fig. 1).

Pathogenicity tests of isolates from outside the flue-cured tobacco area of North Carolina exhibited a similar pattern of variation. Among the foreign accessions, the isolate from Puerto Rico and 1 isolate from Java were pathogenic. The other isolate from Java and the single isolates from India and from Sumatra were nonpathogenic. The isolates from India and Sumatra had been maintained in culture for several years before they were obtained for this study; during this time they may have lost virulence.

Cultural and physiological variation.—In an attempt

TABLE 2.—*Pathogenicity indices following inoculation of 12-in. plants in 6-in. pots with selected isolates of Phytophthora parasitica var. nicotianae*

Isolate	Index in test with indicated variety[a]		Isolate mean[b]	Predicted pathogenicity level[c]
	Bottom Special	Dixie Bright 101		
309	42.7	16.0	29.4	Intermediate
354	15.5	3.2	9.4	Low
373	24.0	0.0	12.0	Low
376B	47.0	26.7	36.8	Intermediate
417	72.0	18.3	45.2	High
423	56.5	27.8	42.1	High
906	24.3	8.5	16.4	Low

[a] Variety Bottom Special–susceptible; variety Dixie Bright 101–moderately resistant. Six plants of each test variety were used. Counts of diseased plants were made at 4-day intervals during 32-day period after inoculation. Classes (corresponding to days after inoculation) and class values were as follows: 4 days–100; 8 days–95; 12 days–90; 16 days–80; 20 days–60; 24 days–40; 28 days–20; 32 days–10.

[b] LSD between isolate means: 5 per cent = 8.1; 1 per cent = 12.0.

[c] The predicted pathogenicity level of the isolates was based on seedling inoculation tests.

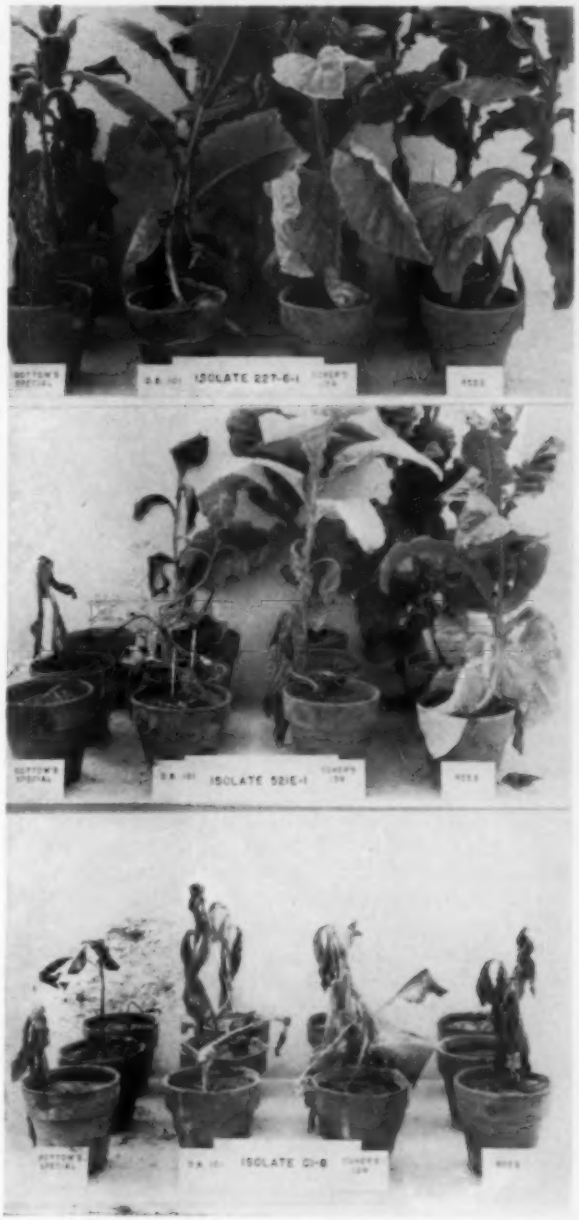

FIG. 1. The effects of a weakly pathogenic isolate (227-6-1) and of 2 highly pathogenic isolates (521E-1 and C1-8) on a susceptible variety (Bottom Special), on 2 resistant varieties (D. B. 101 and Coker 139), and on a USDA resistant breeding line that derived its resistance from *N. plumbaginifolia* Viv. (R55S).

to find morphological and/or physiological characteristics that were either specific for isolates from certain geographic areas or correlated with pathogenicity, comparative studies were made with respect to cultural morphology on various media and to response to hydrogen-ion concentration.

At the outset, it was noted that isolates acquired from the burley-tobacco-producing areas of North Carolina, Tennessee, or Kentucky differed in colony morphology on oatmeal agar from those obtained from the flue-cured-tobacco area of North Carolina. The former produced aerial mycelium in greater abundance and generally grew more rapidly than did isolates from flue-cured-tobacco areas. These differences proved to be consistent without exception and were even more striking on V-8 juice agar than on oatmeal agar. On this medium, isolates from the burley-tobacco area produced smooth, rapidly expanding colonies with definite margins and aerial mycelium, whereas isolates from the flue-cured-tobacco area produced irregular, slow-growing colonies of submerged abruscoid mycelium (Fig. 2, A).

All isolates of the black shank fungus from South Carolina, Pennsylvania, Maryland, Puerto Rico, and Virginia were similar in colony morphology to those from the area where flue-cured tobacco is produced. Representatives of both types were obtained from Florida and Georgia, and a single isolate from California was of the burley type. An isolate from India and isolates from Java and Sumatra acquired through the Centraalbureau voor Schimmelcultures, Baarn, Holland, as *P. parasitica* var. *nicotianae* were much different from any of the isolates from the United States. An isolate of the black shank fungus received directly from Java was identical in cultural morphology with the type characteristic of areas that grow flue-cured tobacco.

A significant difference also was noted between the 2 types of isolates when compared at various pH levels on tobacco-extract agar. The maximum point of growth for burley isolates occurred at a lower pH level than did that for the other type. Of the 2 types, the burley isolates made greater growth at the lower pH levels and less growth at the higher pH levels (Fig. 3).

There was some indication that the buffer mixture used in these tests had an inhibitory effect upon growth of burley isolates. They usually made less radial growth on the buffered medium than did isolates of the other type (Fig. 3). This was not the case on unbuffered media (Fig. 2, A).

Pathogenic and cultural variation among sister single-zoospore isolates.—In pathogenicity tests, a 4-year-old isolate (isolate 906) was found to be weakly pathogenic although it was judged to be "normal" in pathogenicity when originally isolated. It was assumed that isolate 906 had lost virulence while in culture. In an attempt to analyze the nuclear change that had taken place within this isolate, several single-zoospore isolations were made from it and the resulting isolates compared, both in culture and in inoculation tests. Differences in colony morphology and growth rate were apparent, but they were not as striking as differences in pathogenicity among isolates. Mean pathogenicity indices varied from a low of 0.0 (nonpathogenic) to a high of 9.5. Isolate 906-9 was consistently the most pathogenic and isolate 906-8 was never observed to cause disease. This variation was comparable to that found among samples from natural populations.

In further studies, single-zoospore isolates were obtained from other mass-transfer cultures and compared.

When several single-zoospore isolates from isolate 320, which was highly pathogenic (Table 1), were plated out on a V-8 juice agar, little or no cultural variation was apparent. Twenty-five single-zoospore cultures from this isolate were checked for pathogenicity in a 2-replicate experiment on Bottom Special, but there were no significant differences among the cultures.

There were no striking cultural variants among the single-zoospore isolates of 309 and 324, but there was significant pathogenic variation. One isolate from 324 was nonpathogenic, and highly significant differences were apparent among the others. The single-zoospore isolates from 309 did not show extreme variation in pathogenicity as did those from 324 and 906, but there were statistically significant differences among them.

Culture 358, which was a weakly pathogenic isolate from the flue-cured-tobacco area, had the cultural appearance of a burley isolate on V-8 juice agar but that of a flue-cured-tobacco isolate on tobacco-extract agar. Single-zoospore isolates from this culture showed extreme cultural variation. On V-8 juice agar, some of the single-zoospore isolates also resembled the burley isolates, but some were intermediate and one was typical of flue-cured-tobacco isolates (Fig. 2, B). On tobacco-extract agar, all the single zoospore isolates resembled the flue-cured-tobacco isolates, but on this agar those that resembled the burley isolates on V-8 juice agar grew most rapidly (Fig. 2, C). These isolates were not checked for pathogenicity.

Several hundred single-zoospore isolates from the burley and flue-cured-tobacco types were studied. Although considerable intratype variation was noted, no variant represented a change from its parental type to the other cultural type.

Discussion.—The inoculation studies indicate that *P. parasitica* var. *nicotianae* contains pathogenic variants. Furthermore, these pathogenic variants may be characterized by general and arbitrary classes according to their level of pathogenicity, since a given isolate tends to perform consistently in successive inoculation tests. The following evidence indicates that the seedling-inoculation technique by which these class determinations were made is a valid measure of pathogenicity. 1) The relative pathogenicity of the 2 check iso-

lates did not vary in different pathogenicity trials. 2) Isolates determined as high, intermediate, and weak in pathogenicity by the seedling-inoculation technique were rated similarly when used to inoculate large potted plants (Table 2). 3) A given isolate used in 2 or more pathogenicity tests generally was consistent in performance.

There was a positive correlation between the pathogenicity of an isolate on the susceptible varieties and its pathogenicity on resistant varieties, e.g. isolates highly pathogenic on the susceptible Bottom Special were also among the most highly pathogenic on the

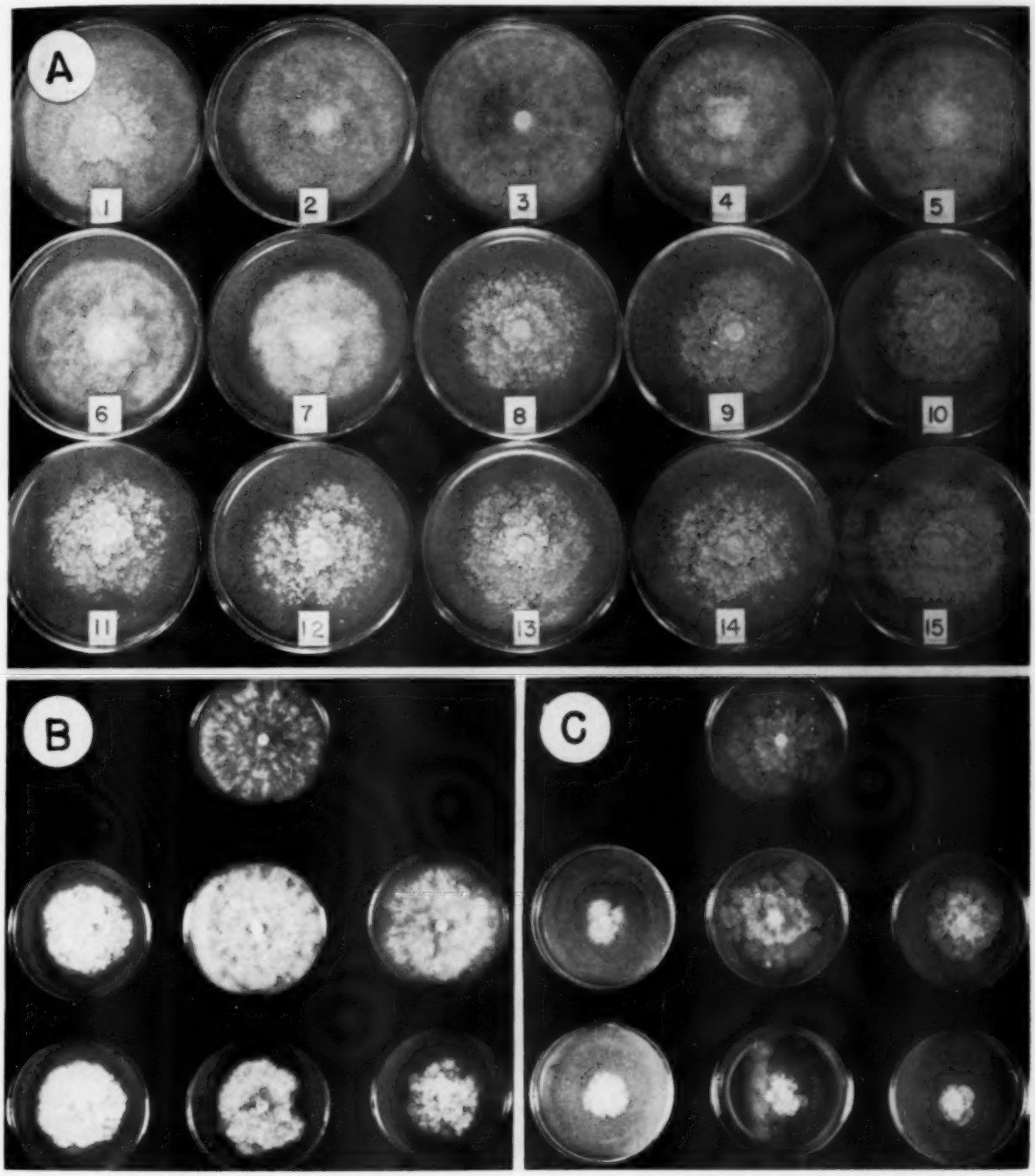

FIG. 2. A) Cultural differences between burley isolates (plates 1–7) and flue-cured tobacco isolates (plates 8–15) on V-8 juice agar. B, C) Growth characteristics of flue-cured tobacco isolate 358 (top) and of its single-zoospore isolates on V-8 juice agar (B) and on tobacco-extract agar (C).

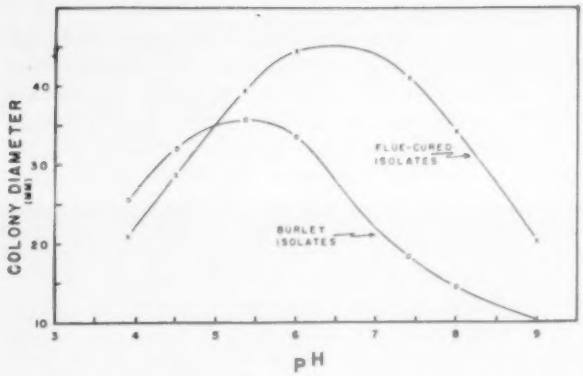

FIG. 3. Curvilinear regression of growth on pH of black shank isolates from flue-cured and burley tobaccos after 6 days on tobacco-extract agar adjusted to various pH levels. The dependent variable (growth) was converted to logarithms, and the regression values obtained were converted to antilogarithms. The curve for the isolates from burley tobacco is the average regression coefficient for 2 isolates, and that for isolates from flue-cured tobacco is the average of 7 isolates.

resistant varieties Dixie Bright 101 and Coker 139; in addition, isolates weakly pathogenic on the susceptible were also weak or nonpathogenic on the resistant varieties. Variation of isolates studied ranged from weakly pathogenic on the susceptible variety to highly pathogenic on the resistant varieties with no natural classes apparent.

On the test varieties of tobacco used in this study, there was no indication of physiologic races of the black shank fungus, although the variety × isolate interaction in some of the inoculation tests was statistically significant at the 1 per cent level. This interaction was created by isolates that were intermediate in pathogenicity on Bottom Special but failed to kill any of the resistant plants. Since a positive correlation is apparent between the performance of all isolates on the varieties used, this interaction is not interpreted as indicating physiologic races. If physiologic specialization exists within this organism, its demonstration will likely require the use of host varieties or species carrying resistance that differs genetically from that of present commercial varieties that are resistant to black shank. The utilization of genetically different sources of resistance preceded the discovery of physiologic races in *P. infestans* (4) and in *P. fragariae* (13).

The existence of pathogenic variants within the black shank fungus appears to be one of the factors contributing to the breakdown of resistant varieties in some fields and may become increasingly important in the future. A hypothesis by which this situation could come about is based upon the concept of adaptive selection in response to an environmental change, a basic theory of population genetics (12).

The resistant varieties now in use have moderate to high resistance against the black shank organism, but they are not immune. Therefore, when such varieties are planted in infested soil year after year, they may serve as a screening mechanism by which the highly pathogenic variants are sorted out and perpetuated. There would be an obvious selection pressure in favor of the highly pathogenic biotypes under such conditions, since they would be more efficient in attacking the resistant variety than would be the intermediate or weakly pathogenic biotypes. As a result, the fungus population in an infested field continuously planted to a resistant variety would change from one containing a mixture of pathogenic biotypes to one comprised largely of the highly pathogenic type. Also, under such conditions, a mutation pressure would be exerted in the direction of higher pathogenicity, e.g. if gene "A," which produced an intermediate or "normal" level of pathogenicity, should mutate to gene "a," which produces a higher level of pathogenicity, the latter would have an immediate selective advantage and would become established in the population to the eventual exclusion of gene "A." Any mutation that improves the chance of survival of its carriers will have the opportunity of becoming established as the "normal" or prevailing condition (14).

Adaptive selection appears tenable as a working hypothesis in partial explanation for the occurrence of a high incidence of disease in varieties resistant to black shank. This is supported by the fact that isolations from these black shank "problem" fields yielded a higher percentage of highly pathogenic strains than did isolations from fields in which resistant varieties had not been grown.

The fact that pathogenic variation occurs within *P. parasitica* var. *nicotianae* and the likelihood that adaptive selection can take place where resistant varieties are grown continuously in infested fields have some very important implications. 1) The present moderately resistant varieties may eventually lose their usefulness because of the widespread increase of highly pathogenic strains. 2) The plant breeder in developing new and more highly resistant varieties must be thorough in testing his breeding material; it must be subjected to the most highly pathogenic strains to enable selection of host genotypes with highest resistance.

The cultural and physiological differences between isolates from the flue-cured and burley areas were sufficiently clear-cut to warrant the designation of the 2 types as distinct cultural strains, viz., the flue-cured-tobacco strain and the burley strain. If a different substrate and/or buffer system had been used, the hydrogen-ion response for the 2 groups may have been different; nevertheless, these pH data show a basic physiological difference between the 2 groups.

The existence of 2 distinct strains suggest independent origins. It is possible that the black shank fungus could arise from the closely related fungus, *P. parasitica*, either through mutation or hybridization. It is not likely, however, that independent origins have occurred frequently enough to explain the occurrence of new outbreaks of the black shank disease in widely separated areas within or between states as suggested by Johnson and Valleau (7). The existence

of 2 compatibility groups (2, 7) does not necessarily indicate as many independent origins, since they could easily arise from existing bisexual thalli by mutation or segregation. It does not seem logical to assume that the black shank fungus has arisen on such frequent occasions. If so, 1) much more cultural diversity would be expected to occur than is apparent; 2) the burley and flue-cured-tobacco strains would not be expected to occur in distinct geographic areas; and 3) the black shank disease should have been present in this country long before its discovery. This cultural study indicates, however, that this organism has arisen on at least 2 separate occasions, each giving rise to a distinct cultural strain. This hypothesis is supported by the following: 1) no interstrain variants were found among single-zoospore isolates from a given strain, 2) the 2 strains differ in mating compatibility (2, 7), and 3) they differ physiologically. These basic differences between the 2 strains indicate that one is not a simple mutant of the other.

An extensive world survey of the black shank fungus population would be necessary before the point or points of origin of the 2 strains could be postulated; however, within the United States it is likely that the Georgia-Florida area is the infestation center from which this pathogen has been spread to other tobacco-producing areas. The flue-cured-tobacco strain was introduced into the North Carolina flue-cured area from which it has been spread into Virginia and South Carolina. The burley strain became established in Kentucky and Tennessee and has recently been spread into the burley-producing area of western North Carolina.

The variation among single-zoospore isolates from a single culture demonstrates the variation potential of the black shank fungus. In the case of isolate 906, it was assumed that the variants arose through mutation in culture. It is not likely that such genetic diversity could exist in nature within a single thallus, since infection is commonly initiated by means of uninucleate zoospores; consequently, it probably did not possess this variation when isolated. In order to eliminate the possibility that this mass-transfer isolate was composed of a mixture of 2 or more thalli, single hyphal-tip cultures were made. Single zoospore isolates subsequently obtained exhibited the same pathogenic and cultural variation as did the initial single-zoospore isolates. Thus, the parent 906 was heterocaryotic, since genetically diverse nuclei did reside in a common cytoplasm. It appeared that the genetic factors governing the weakly pathogenic condition were dominant in this heterocaryotic system, since the parent was weakly pathogenic, although it contained nuclei carrying genes for high pathogenicity. The extremely weak pathogenic type nucleus was in the minority (1:5), nevertheless, it dominated the phenotypic expression of pathogenicity. This is similar to the situation found by Beadle and Cooradt (3) in their work with biochemical heterocaryons of *Neurospora*, in which certain genes exhibited full dominance when in the ratio of 1 dominant to 17 recessive.

Single-zoospore isolates from highly pathogenic parents gave rise to few cultural or pathogenic variants; however, weakly pathogenic isolates always yielded cultural and pathogenic variation when single-spore isolations were made. This indicates that the loss in pathogenicity often observed in culture is due to an unstable genetic condition that gives rise to weakly pathogenic or nonpathogenic genotypes that are expressed phenotypically.

The nonpathogenic single-zoospore isolates obtained could not be considered *P. parasitica* var. *nicotianae* according to the recognized taxonomic criterion that stipulates pathogenicity on tobacco in distinguishing it from *P. parasitica* (16). If isolates of the black shank fungus can mutate to *P. parasitica*, the back mutation must also be possible by which the latter could become *P. parasitica* var. *nicotianae*.

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INDEPENDENCE IN THE ACTION OF THREE GENES CONDITIONING STEM RUST RESISTANCE IN RED EGYPTIAN WHEAT [1]

W. Q. Loegering and J. R. Geis [2]

SUMMARY

The wheat varieties Red Egyptian and Chinese and 3 substitution lines of chromosomes VI, XIII, and XX of Red Egyptian into Chinese were inoculated with 3 cultures of *Puccinia graminis* f. sp. *tritici* and then held under 5 different environmental conditions. In 13 of 15 combinations of fungus culture and environmental condition, one or the other of the 3 substitution lines developed an infection type almost identical to that produced on Red Egyptian. This indicates that only one of 3 genes in Red Egyptian was effective in conditioning resistance under a specified combination of fungus culture and environmental condition. In the other 2 combinations, Red Egyptian was considerably more resistant than any one of the substitution lines, indicating that its resistance in these cases was due to an interaction between at least 2 genes.

Red Egyptian wheat, C. I. 12345, has been used as a parent in breeding wheats resistant to stem rust. Knott and Anderson,[3] and Sears, Loegering, and Rodenhiser[4] have shown that it has at least 3 genes for resistance—Sr6, Sr8, and Sr9 on chromosomes XX, VI, and XIII, respectively. The availability[5] of the 3 chromosome substitutions into Chinese wheat[6] that carry resistance from Red Egyptian wheat made possible a comparison between the stem rust reactions of the 3 substitution lines and the reaction of Red Egyptian.

Five lines of wheat were used in the study and are listed in Table 1. Chinese (C. I. 6223) was included because it is the background variety for the 3 chromosome substitution lines. Three cultures of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. were used as follows: culture 15B-51A (Race 15B) and culture 56-51A (Race 56), collected prior to 1951, but of unknown origin, and culture 17-53B (Race 29), collected at Mexe, Hidalgo, Mexico, Dec. 19, 1953. Five pots of seedling plants of each line were inoculated with each of the 3 cultures and, after removal from the inoculation chamber, were distributed among 5 different sets of conditions of temperature and light as given in Table 2. These 5 sets of conditions are designated throughout this report by the average temperatures. The tests were made under these conditions with more than 1 culture because it had previously been observed that temperature, particularly, affected the reaction of seedlings of Red Egyptian to some races of the stem rust fungus. Green and Johnson[7] reported this to be true with adult plants also.

Table 1.—*Lines of wheat inoculated with 3 cultures of Puccinia graminis f. sp. tritici*

Line	No.	Source	Genotype
Red Egyptian	C. I. 12345	Egypt	Sr6 Sr8 Sr9 [a]
R. E.-VI	P55-106.6b-2	E. R. Sears	sr6 Sr8 sr9
R. E.-XIII	P53-113.6b-16	E. R. Sears	sr6 sr8 Sr9
R. E.-XX	P55-120.6b-2	E. R. Sears	Sr6 sr8 sr9
Chinese	C. I. 6223	China	sr6 sr8 sr9

[a] According to Knott and Anderson (Footnote 3).

Table 2.—*Conditions under which 5 lines of wheat were grown after inoculation with different cultures of Puccinia graminis f. sp. tritici*

Temperature in °F		General conditions	
Average	Range	Location	Light period
78	72–90	Greenhouse [a, b]	Normal
71	65–88	Greenhouse [a]	Normal
68	67–69	Light room [c]	18 Hours
59	50–85	Greenhouse [a]	Normal
55	50–60	Light room [c, d]	12 + 2 Hours

[a] Normal fluctuations in light and temperature.
[b] High humidity maintained by spray.
[c] Fluorescent and incandescent lights provided 1000 foot-candles at plant level. Temperature controlled ±1°F.
[d] Light period of 12 hours at 60° followed by 5 hours dark, 2 hours light, and 5 hours dark at 50°.

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TABLE 3.—*Reaction of seedlings of 5 lines of wheat to 3 cultures of Puccinia graminis f. sp. tritici under 5 environmental conditions*

Culture	Line	Reactions[a] at indicated environmental conditions[b] in 3 tests															
		78°F			71°F			68°F			59°F			55°F		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
17-53B	Chinese	4—	3+4—	4—	4	4	4	3++	4—	3+	3+	3+	3++	3+	3+	3+
	R. E.-VI	4—	3+4—	4	4	4	4	3+	3+	3+	3++	3	3++	3	3+	3
	R. E.-XIII	2=	2=	2≡	2—	2+3[c]	2	3—	0;2=	2=	2—3=	3≡	3=	2≡	2=	2≡
	R. E.-XX	4—	3+4—	4—	4	4	4	3+	4—	3+	3 3+	3+	3+	3	3+	3
	Red Egyptian	2≡	2≡	0;	2—	2	3≡	2 3—	2≡	2=	2 3=	1	3=	2≡	2=	2≡
56-51A	Chinese	3+	3+4—	4	4	4	4—	3+	4—	3+	3+	3+	3+	3	3+	3
	R. E.-VI	3	3—	3—	3—	3—[c]	3—[c]	3—	3—	3	3—	3—	3—	3=	3—	3—
	R. E.-XIII	2	3—[c]	0;2=	2 3—	3—[c]	2	3—[c]	2—3=	2≡	3—[c]	3=	3=	2—3=	2=	3≡
	R. E.-XX	3+	3+	4	0;	0;1	0;	0;	0;	X=	0;	0	0	0	0;	0
	Red Egyptian	2=	2=	2=	0;	0;	0;	0	0;	0;	0	0	0	0	0	0
15B-51A	Chinese	3+	3+4—	4	4	4	4	3 3+	4—	3+	3+	3+	4—	3—3	3+	3
	R. E.-VI	3—	3	3—	3	3	3	3=	2—3≡	3—	3=	3—[c]	3—	0;3=	0;3=	0;3=
	R. E.-XIII	X+	3+	3+[c]	X+	4—	X+	3 3—	3+	X+	3 3+[c]	3	3+	3=3	3 3+	3—3
	R. E.-XX	3+	3+	3 3+	X	X	X	0;1+3—	X+	X+	0;	0;	0;	0;1	0;1—	0;
	Red Egyptian	3—3[n]	3	X	X=	X=	X—	X=	0;	3—[c]	0;	0;	0;1	0;	0;	2≡

[a] Reactions are recorded as infection types according to the method of Stakman, Levine, and Loegering (Footnote 8).
[b] The conditions are designated by the average temperatures. See Table 2.

The experiment was repeated 3 times at 5-day intervals during March and April, 1957. Data were taken according to infection type.[8] The results of the 3 tests are presented in Table 3.

R. E.-VI and R. E.-XX were as susceptible as Chinese to culture 17-53B (Race 29), whereas Red Egyptian and R. E.-XIII were resistant under all conditions. Sears, Loegering, and Rodenhiser[4] reported that R. E.-XIII was more resistant than Red Egyptian to culture 17-53B, but this was not confirmed in the present work.

R. E.-XX was susceptible to culture 56-51A (Race 56) at the highest temperature, but Red Egyptian and R. E.-XIII were moderately resistant. Both Red Egyptian and R. E.-XX were very resistant to culture 56-51A at the 4 lowest temperatures. R. E.-VI was intermediate and R. E.-XIII was moderately resistant in reaction at all temperatures.

Chinese was susceptible and R. E.-XIII was moderately susceptible to culture 15B-51A (Race 15B) under all conditions. R. E.-XX was susceptible at the highest temperature. Red Egyptian and R. E.-XX were highly resistant at the 2 lowest temperatures, Red Egyptian was resistant at 71° and 68°F, and R. E.-VI was resistant at 55°F. An intermediate reaction developed on Red Egyptian and R. E.-VI at 78° and also on R. E.-VI and R. E.-XX at 71° and 68°F.

On the basis of the above data, the variability in the reaction of Red Egyptian wheat to different cultures of the stem rust fungus under different environmental conditions can be explained. Red Egyptian in all tests varied in reaction only slightly, if at all, from that of one or the other of the substitution lines. In 13 of the 15 combinations of race and environmental condition, the differences in reaction were so small that they have little or no significance. This indicates that the reaction of Red Egyptian under a given set of conditions is usually controlled by 1 gene; the particular gene involved depends on the race with which the plants are inoculated and on the environmental conditions under which rust development takes place. Its resistance to culture 17-53B is due to gene Sr9 under all conditions tested. Resistance to culture 56-51A at the high temperature is due to the presence of gene Sr9, whereas at the 4 lower temperatures it is due to gene Sr6. Resistance to culture 15B-51A at 59° and 55° is due to gene Sr6, whereas resistance at 78° appears to be due to gene Sr8. At 71° and 68°, Red Egyptian is considerably more resistant than any of the substitution lines, indicating that its resistance in these 2 cases results from an interaction, perhaps of genes Sr6 and Sr8.

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A MAJOR GENE LOCUS FOR COMPATIBILITY IN COCHLIOBOLUS HETEROSTROPHUS [1]

R. R. Nelson [2]

SUMMARY

Studies on the segregation of ascospore isolates of *Cochliobolus heterostrophus* indicate that compatibility is controlled by a single major gene locus. Of the 995 monoascosporic isolates studied, 484 belonged to compatibility group *A* and 511 to compatibility group *a*. Isolates studied in ascus progenies also segregated 1:1 for compatibility. Studies of monoconidial isolates obtained from diverse geographic areas indicate that the compatibility group factor is segregated in a 1:1 ratio in nature. Recent crosses have yielded a monoascosporic culture that produces sterile asci when selfed and another that is self-fertile. The behavior of these isolates, together with the marked differences in degree of intergroup and intragroup fertility among monoconidial isolates, indicates that the sexual process in *C. heterostrophus* is controlled by a number of gene loci.

Southern leaf spot, caused by *Helminthosporium maydis* Nisik. & Miyake, is one of the most important diseases of corn in the southeastern United States. The disease frequently becomes widespread throughout this area where high temperatures and high relative humidity prevail during a greater part of the growing season. In conjunction with a projected program of breeding for resistance to this disease, it was considered important to conduct basic investigations on the genetic behavior and the genetic variability of the pathogen, particularly with respect to its pathogenic potentialities. Prior to this study, the ascigerous stage of the fungus, *Cochliobolus heterostrophus* Drechs., never had been reported as being obtained in culture on agar media, thus precluding genetic analyses.

The early phases of this research were concerned with the materials and techniques most suitable for the culture of the perfect stage on artificial media and with a preliminary analysis of the basic mechanisms controlling the sexual process.[3] It was shown that *C. heterostrophus* is heterothallic in that monoconidial and monoascosporic isolates are self-sterile and cross-fertile. Furthermore, the species comprises 2 distinct compatibility groups, designated as *A* and *a*, all isolates exhibiting complete intergroup fertility; however, certain monoconidial isolates of the same compatibility group are fertile when mated with one another, although the occurrence of intragroup fertility is rare and no 2 isolates of the same group consistently produce fertile perithecia when paired with each other.

Further research has been concerned with the inheritance of factors for compatibility, as well as with the nature and mechanism of variability in the degree of compatibility among isolates. The progress of those studies is reported herein.

MATERIAL AND METHODS.—The materials and methods used in these studies were similar to those described previously.[3] Isolates were mated by placing small pieces of mycelium on opposite sides of a section of sterile corn leaf placed in the center of a Petri plate containing Sach's nutrient agar. All matings were incubated continuously at 24° C.

The following techniques were employed in determining the factors governing the inheritance of compatibility. Cultures derived from single ascospores were obtained from crosses of known compatible isolates, both conidial and ascosporic. Each monoascosporic isolate thus obtained was mated to both parental lines and to tester lines (one *A* and one *a* isolate) to determine its compatibility group. The segregation of isolates into compatibility groups *A* and *a* was determined for each cross. Matings were considered compatible if ascospores were produced.

RESULTS.—Four hundred fifty-five monoascosporic isolates obtained from 40 different conidial matings were studied for their compatibility reactions. The number of ascosporic isolates obtained from any single cross ranged from 1 to 112. Of the total, 220 isolates belonged to group *A* and 235 to group *a*. The segregation of isolates obtained from any 1 specific cross never deviated appreciably from the 1:1 ratio. For example, the 112 monoascosporic isolates obtained from 1 cross segregated into 52 *A* and 60 *a* isolates.

Of 540 monoascosporic isolates obtained from 6 different ascospore matings, 264 isolates belonged to compatibility group *A* and 276 to group *a*. The segregation of ascosporic isolates obtained from all conidial and ascosporic crosses totalled 484 *A* and 511 *a*.

Segregation for compatibility also was computed in ascus progeny studies. The value of investigating the progeny of individual asci in *C. heterostrophus* is somewhat limited, since it is not possible, with the techniques now known, to determine when reduction division occurs, and serial isolation of ascospores is precluded. Asci in *C. heterostrophus* contain 1 to 8

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[3] Nelson, R. R. 1957. Heterothallism in Helminthosporium maydis. Phytopathology 47: 191-192.

ascospores in a tightly coiled helix, the coiling being from right to left, from the base of the ascus to its apex. Asci containing from 1 to 8 spores may be found in 1 perithecium. Approximately 85 per cent of the asci contain from 3 to 6 ascospores.

Poor germination of ascospores usually was encountered in these studies. Attempts to increase germination by the use of a number of media varying in nutrients and pH were not successful. Germination of spores obtained from different crosses ranged from 0 to approximately 50 per cent.

Despite this poor germination, it was possible to study 36 complete ascus progenies. The ascus groups were obtained from 6 conidial crosses involving 9 isolates and from 11 ascospore matings involving 10 isolates. Segregations for compatibility in the ascus groups are listed in Table 1. The segregation of ascosporic isolates of all progenies totalled 79 *A* and 77 *a*.

Other data support these findings. Thirty-four monoconidial isolates were used extensively in the mating studies here reported. The isolates were obtained from widely scattered areas in Virginia, Maryland, North Carolina, South Carolina, Georgia, and Florida. A single isolate from Columbia, South America, also was included. It was considered that the isolates constituted a representative sample of the pathogen and should have afforded a broad genetic base. Nineteen of the isolates belong to group *A* and 15 to group *a*. Furthermore, studies of crosses of wild-type isolates with albino isolates yielded approximately equal numbers of wild type *A*, wild type *a*, albino *A*, and albino *a* isolates.

The possibility that multiple alleles exist at the same locus was considered. Simple back matings of ascospore progenies to parental lines would not disclose the presence of such factors. Monoascosporic isolates, obtained from crosses of conidial and/or ascopore lines, were mated to tester lines to determine their compatibility group. *A* isolates from 1 cross then were mated with *A* isolates obtained from other crosses involving different *A* and *a* parents. Similar matings were made among *a* isolates. If multiple alleles were present at the locus for compatibility, fertile perithecia would be obtained from $A \times A$ and/or $a \times a$ crosses. Such fertile matings should occur consistently whenever such isolates are paired.

A and *a* isolates obtained from 5 crosses involving 9 different parent lines were paired in all possible intragroup combinations. No fertile crosses have resulted from the more than 2100 different matings. These preliminary results suggest that multiple alleles are not present at the locus for compatibility. Additional pairings involving a wider genetic and geographic base are now being screened.

Table 1.—*Segregation for compatibility in complete ascus progenies of Cochliobolus heterostrophus*

No. of spores per ascus	No. of cultures per ascus possessing indicated compatibility		No. of asci studied
	A	*a*	
1	1	0	1
2	1	1	4
	0	2	2
	2	0	1
3	1	2	2
	2	1	3
4	2	2	5
	1	3	2
5	2	3	2
	3	2	4
6	3	3	1
	4	2	3
7	3	4	3
	4	3	1
8	4	4	2
Total no. of asci			36
Total no. of cultures with indicated compatibility	79	77	

Discussion. — The results summarized herein strongly indicate that compatibility in *C. heterostrophus* is controlled by a single major gene locus. It is realized that the gene for compatibility is only a single link in the sexual process of the fungus. Modifying or secondary genes undoubtedly influence the gene locus for compatibility, whereas other gene loci control additional steps in the sexual mechanism. The phenomenon of intragroup fertility[3] could be explained not only by the presence of multiple alleles but also by the presence of additional gene loci.

A recent cross of 2 monoconidial lines yielded a monoascosporic culture, designated as isolate 90, which produces sterile asci when selfed. When crossed with other isolates, isolate 90 is heterothallic and mates only with isolates in compatibility group *A*. No reaction occurs when isolate 90 is paired with group *a* isolates, not even the production of perithecia with sterile asci, of which isolate 90 is capable of when selfed.

A cross of 2 heterothallic monoascosporic isolates recently produced a culture, designated as isolate 15, that forms typical perithecia with fertile ascospores when selfed. All ascospore progeny derived from the self-fertile isolate are heterothallic. The ability of isolate 15 to form ascospores can be maintained repeatedly through mycelial transfers from the original culture or subcultures thereof.

The marked differences in degree of intergroup and intragroup fertility among monoconidial isolates suggests definite qualitative or quantitative differences in genetic make-up. The role that these factors play in the completion of the sexual process and their relationship to the major gene locus for compatibility are being investigated. Studies of this nature should afford new evidence concerning the genetic mechanisms of homothallism and heterothallism.

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HELMINTHOSPORIUM SATIVUM, A DESTRUCTIVE PATHOGEN OF BLUEGRASS [1]

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SUMMARY

Helminthosporium sativum Pam., King & Bakke was found to be the principal disease organism affecting bluegrass turfs in eastern Nebraska in 1956. The symptoms of the disease were found to vary greatly with temperature and moisture. Under experimental conditions of continuous moisture and temperatures of 20°–25°C, the disease was manifested as a leaf spot; at 30°–35°C and continuous moisture, blighting of the leaves and killing of entire plants occurred; at 30°C and alternating moist and dry conditions, leaf spot symptoms developed but blighting did not occur. These various symptom expressions were correlated very well with those occurring in lawns.

Results from greenhouse studies and the findings from surveys of diseased lawns suggest that control might be gained through fungicidal sprays and employment of those cultural operations that permit the greatest amount of drying of the foliage without being harmful to the maintenance of a fine turf.

There has been a considerable amount of dying-out of large, irregularly shaped areas in well-cared-for bluegrass (*Poa pratensis* L.) lawns during midsummer of the past several years in eastern Nebraska. In the summer of 1956, isolations from diseased plants yielded primarily *Helminthosporium sativum* Pam., King & Bakke.

H. sativum has been reported as a pathogen of bluegrass;[3, 4] however, other reports list bluegrass as resistant to this organism.[5, 6] It has not been previously considered as a seriously destructive pathogen of bluegrass lawns, although in recent years it has been observed to cause considerable destruction of bentgrass (*Agrostis palustris* Huds.) turfs.[7]

Preliminary tests.—Routine inoculation tests with *H. sativum* isolated from bluegrass proved the organism to be very pathogenic. Inoculations were made by spraying the plants with an aqueous spore suspension or with a suspension of spores and mycelium.

The inoculated plants were placed in a chamber at 100 per cent relative humidity for several days, after which time they were transferred to a greenhouse. Depending upon temperature, symptoms in the form of leaf spotting or blighting appeared in 5–6 days.

Effect of temperature on symptom expression.—Groups of 10 pots of inoculated bluegrass were placed at 100 per cent relative humidity at each of the following temperatures: 20°, 25°, 30°, and 35°C. After 7 days, they were transferred to a greenhouse having low humidity and a temperature of about 26°C. Infection occurred at all temperatures, but there was considerable variation in symptom expression. The readings made 12 days after inoculation were as follows: at 20°, considerable leaf spotting but no leaf blighting; at 25°, considerable leaf spotting and a slight amount of leaf blighting; at 30°, rather severe leaf blighting and a slight amount of leaf spotting; and at 35°, very severe blighting and killing of about 50 per cent of the plants but no leaf spotting. The leaf spots first appeared as purple areas, the centers of which dried out and turned brown. They are similar to the spots caused by *Helminthosporium vagans* Drechs., a well-known pathogen of bluegrass.

The blighting was manifested by a sudden collapse and drying out of the leaf blades. Following blighting, the leaves blanched to a straw color in a few days.

Time required for infection.—Excised leaves were "painted" with a heavy suspension of spores and then placed at 100 per cent relative humidity at 25°C. After periods of 2, 4, 6, 8, and 10.5 hours, leaves were removed and washed in running water; simultaneously, the leaf surfaces were rubbed with a fine brush. After the leaves were microscopically examined for freedom from nongerminated spores, they were placed in the humidity chamber for 5 days at which time they were observed for lesion development. No lesions developed on the leaves on which spores were allowed to remain for 2, 4, or 6 hours. There were 7 and 12–14 lesions per 3-in. section on leaves that were exposed to spores for 8 and 10.5 hours, respectively. On the check leaves, on which spores remained for 5 days, lesions were so numerous that they coalesced.

These data indicate that a period of 8–10 hours is required for a substantial amount of infection to occur at 25°C.

Effect of alternating low and high humidity.—Fourteen pots of bluegrass were sprayed with a spore suspension and placed in a chamber maintained at 100

[1] Accepted for publication July 29, 1957.

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[2] Extension Plant Pathologist and Research Assistants, respectively, University of Nebraska.

[3] Sprague, R. 1950. Diseases of cereals and grasses in North America. Ronald Press, N. Y. 538 p.

[4] Weiss, F. 1950. Index of plant diseases in the United States. Part III. Gramineae. U. S. Dept. Agr. Plant Disease Survey Special Publ. 1, p. 383-529.

[5] Christensen, J. J. 1922. Studies on the parasitism of Helminthosporium sativum. Minn. Univ. Agr. Expt. Sta. Tech. Bull. 11, 42 p.

[6] Howard, F. L., J. B. Rowell, and H. L. Keil. 1951. Fungus diseases of turf grasses. Rhode Island Agr. Expt. Sta. Bull. 308, 56 p.

[7] Klomparens, W. 1953. A study of Helminthosporium sativum Pam., King & Bakke as an unreported parasite of Agrostis palustris Huds. Ph.D. Thesis. Mich. State Univ. 77 p.

per cent relative humidity and 30°C. After 48 hours (8 a.m.), 7 pots were transferred to a greenhouse at 30 per cent relative humidity and 26°C; 9 hours later (5 p.m.), they were returned to the humidity chamber. This procedure was repeated every day for 5 days. The remaining 7 pots were kept continuously in the humidity chamber.

On the plants kept continuously at 100 per cent relative humidity, no symptoms appeared until the third day, when patches of mycelial growth were visible on the surfaces of the leaves. These patches had increased by the fourth day, and the areas under them were water-soaked. By the fifth day, the water soaking was general in areas of heavy surface growth; the water-soaked leaves had collapsed by the sixth day and were dead by the seventh day. Symptom development was delayed on plants alternated between moist and dry conditions, and the leaves did not blight; instead, necrotic spots developed. No symptoms developed until the fifth day, when a small amount of mycelial growth was visible. Small necrotic spots had developed in 6 days, and many large necrotic spots had developed by the seventh day.

Spore germination and infection.—Spore germination and infection were observed on leaves of bluegrass. Spores of *H. sativum* began to germinate in bipolar fashion in 30–45 minutes. Germ tubes penetrated either through a stoma or by growing down between surface leaf cells. A bulbous structure about twice the diameter of the germ tube always formed at the point of entry, whether penetration was direct or through a stoma.

The effect of certain fungicides on control.—The fungicides captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide), dichlone (2,3-dichloro-1,4-naphthoquinone), zineb (zinc ethylenebis[dithiocarbamate]) and copper arsenate were tested for control of *H. sativum*. Groups of 10 pots of bluegrass were sprayed with a heavy spore suspension of *H. sativum* and then sprayed with one of the above-mentioned chemicals. After treatment, the pots were placed at 100 per cent relative humidity and 25°C for 7 days, then transferred to a greenhouse where disease development was recorded 3 days later. The fungicides captan, dichlone, and zineb gave good control whereas copper arsenate did not (Table 1).

Pathogenicity on other grass hosts.—The pathogenicity of the culture of *H. sativum* isolated from bluegrass was tested on barley, wheat, rye, and corn. The organism readily attacked these species, causing typical Helminthosporium blight symptoms.

DISCUSSION.—*Helminthosporium sativum* has not heretofore been reported as a major pathogen of bluegrass. There are several possible reasons why it recently has become so destructive. Perhaps a new strain of the pathogen is involved; however, *H. sativum* has been noted before on bluegrass and perhaps the recent marked changes in cultural practices for turfs has created the proper conditions for its development. It has become customary to clip the grass with power mowers to approximately 3 in. in length and to leave the clippings. In time, a 2- to 3-in. mulch is created. This type of cultural practice has several salient features that are conducive to the development of *H. sativum* in the lawn. As a consequence of the use of a power mower, conidia of the pathogen may be widely distributed over the lawn from an initial infection spot. Clumps of matted clippings create an excellent humidity-chamber effect. The disease frequently has been observed to have spread out into the turf from these clumps. Further, the heavy mulch acts as an excellent moisture retainer. Continuous moist conditions so provided would enhance disease intensity. Even though weather conditions may be dry, such a mulch often is kept wet by frequent irrigation. It is possible the pathogen has been present in bluegrass lawns for years in this area, and the damage caused may have been erroneously attributed to other diseases with similar symptoms.

TABLE 1.—*Disease ratings of greenhouse-grown bluegrass inoculated with Helminthosporium sativum and then sprayed with various fungicides*

Fungicide	Concentration lb./gal.	Disease rating*
Captan (50%)	0.04	0.2
Dichlone (50%)	0.02	0.3
Zineb (65%)	0.01	0.8
Copper arsenate (91%)	0.1	2.0
None		3.1

* Disease intensity was measured as follows: 0 = no disease, 1 = few leaf spots, 2 = numerous leaf spots, 3 = moderate blighting, 4 = severe blighting, and 5 = completely necrotic.

The variation obtained in symptomatology as affected by temperature and moisture was similar to that observed in the lawns. In midsummer, during or following prolonged periods of moist conditions and high temperatures, the disease is manifested by a rapid fading-out of irregularly shaped areas of variable size in the lawns. Similarly, leaves were blighted and entire plants were killed in greenhouse tests made at temperatures of 30°–35°C and continuous high relative humidity. Temperatures such as these or higher are common summertime temperatures in Nebraska. At lower temperatures, a leaf spot symptom resembling that caused by *H. vagans* occurred in the greenhouse but did little damage. This leaf spot symptom is common in the lawns in September and October. The observation that alternate dry and moist conditions at high temperatures, which resulted in a predominance of the leaf spot symptom, explains in part why the destructiveness of this disease subsides during the drier portions of the summer.

The control of this disease probably lies in the use of fungicides and of particular cultural practices. Removal of clumps of matted grass following mowing and the spacing of irrigation to permit drying of the foliage and of the mulch between irrigations would appear to be effective measures.

The fact that zineb, dichlone, and captan gave good

control in the greenhouse is indicative that control of the disease in lawns may be effected through the use of fungicides. Possibly Acti-dione (cycloheximide), which has been reported[7] as controlling *H. sativum* in bentgrass, may also be effective against this pathogen in bluegrass turfs.

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A SEVEN-DAY INSTRUMENT FOR RECORDING PERIODS OF RAINFALL AND DEW [1]

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SUMMARY

An instrument for recording periods of rain and dew for 7 days has been developed. A shaft extends from a hygrothermograph through the rainproof top of a ventilated box and connects to an arm holding a pencil. The pencil makes a circle on a ground-glass plate on top of the box as the hygrothermograph drum revolves. Pencils are used that make dark lines on wet ground-glass surfaces and light, indistinct lines on dry surfaces. This instrument provides data on the time of occurrence and duration of wet periods from dew or rain for a period of 7 days.

Equipment to record periods that plants are wet from dew or rain is needed for climatological studies of plant diseases. An instrument to record the duration of dew deposits has been described by Taylor.[2] His device is based on the use of a clock to move a ground glass plate that is marked by an indelible pencil when the surface of the plate is wet. This technique is reliable, but 2 improvements are suggested to make a more versatile instrument. These are 1) a 7-day clock to provide weekly data and 2) a pencil that will record for 1 week without dissolving or smudging. An instrument has been developed incorporating these improvements.

The basic components for a 7-day rain-dew recorder (Fig. 1) are 1) a hygrothermograph, 2) a ventilated box with a rainproof roof, 3) a glass plate with a ground surface, and 4) a bronze shaft and arm connecting the hygrothermograph to a pencil.

The connection of the shaft to the hygrothermograph drum is made by an aluminum disk (Fig. 2, A) having a ridge that fits against the inside of the drum. A cut is made in the ridge to allow space for the chart clip. This clip acts as a key to insure that the disk revolves with the drum.

A bronze shaft, ¼ in. in diameter and divided into 2 parts, extends from the disk through the top of the box. The lower part, which is 2 in. long, is threaded through a hole in the center of the aluminum disk and tightened with a nut. A universal joint (Fig. 2, B) is fastened to the other end to facilitate connecting the upper and lower parts of the shaft by means of a removable pin (Fig. 2, C).

The upper portion of the shaft is 13 in. long and passes through the roof of the box by means of a bronze tube of slightly greater diameter. The uppermost end of the shaft is connected to a horizontal arm, 7¾ in. long, by a hinge joint that permits free vertical movement of the arm. The pencil is inserted in a compass fastened to the outer end of the arm. The drum, shaft, pencil arm, and compass move together as a unit, completing 1 revolution per week.

Two precautions are taken to protect the hygrothermograph from moisture. To prevent water from entering via the shaft, a rubber collar with a broad rim is attached to the rod just above the point of emergence from the tube in the roof of the box. To exclude rain spray, a shield made of cardboard coated with wax is placed over the hygrothermograph, covering all portions except the sensitive elements. A narrow slot in the top of the shield allows the shaft to pass through.

Glass plates, 14 in. in diameter, 3/16 in. thick, and with a ground-glass surface, are used to record the data. Dew formation on the plates occurs about the same time as on leaves, provided that the glass surface is at the same level as the leaves. A hole was drilled in the center of each plate so that it could be placed over the tube protruding from the box. The plates are tilted slightly for drainage of excess water.

Numerous trials were made to find a satisfactory pencil. The best results were obtained with Paradise No. 114 pencils,[3] which make clear heavy lines on wet ground-glass surfaces and light indistinct lines on dry surfaces. Typical markings on a plate are shown in Figure 3. The points of Paradise No. 114 pencils, however, will not persist for an entire week during periods of heavy daily rainfall, such as occur in many tropical areas. For these conditions, 2 other pencils, Waterproof[3] and Weatherproof No. 6639,[4] are satisfactory. The marks of these pencils are less distinct than those made by Paradise No. 114, but the points are more durable. The dark lines made by the 3 different pencils are not smudged or removed by dew or rain but can be washed off with a brush and scouring powder.

To determine the number of hours represented by each mark on a plate, a calibrated chart (Fig. 4) is

[1] Accepted for publication July 15, 1957.

[2] Taylor, C. F. 1956. A device for recording the duration of dew deposits. Plant Disease Reptr. 40: 1025-1028.

[3] Venus Pen and Pencil Co., Hoboken, N. J.

[4] Eberhard Faber Pencil Co., Crestwood, Wilkes-Barre, Pa.

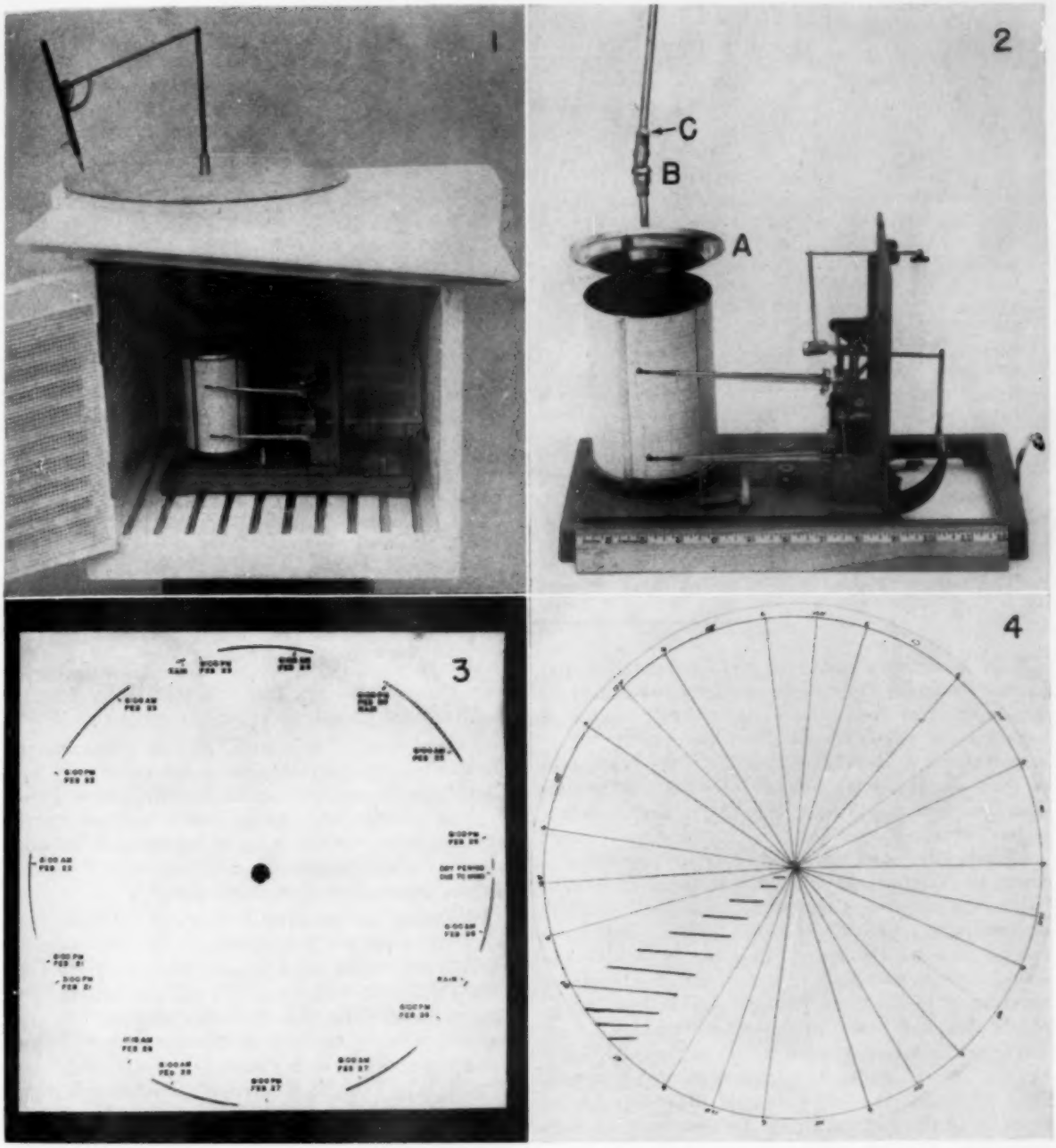

FIG. 1–4. FIG. 1. The main assembly of a 7-day rain-dew recorder.—FIG. 2. A modified hygrothermograph. A) Aluminum disk cut to allow space for chart clip. B) Universal joint for connecting upper and lower parts of shaft by means of a removable pin (C).—FIG. 3. Ground-glass plate of a 7-day rain-dew recorder tested at Mayaguez, P. R., from 3:00 p.m., February 21 to 11:15 a.m., February 28, 1957. A Paradise pencil No. 114 was used. This plate is labeled to illustrate where the 6-hour radii on the time chart (Fig. 4) would appear when the plate is superimposed. This record shows a long wet period during each night and 2 brief daytime showers.—FIG. 4. A chart calibrated at 6-hour intervals for conversion of rain and dew data on glass plates to units of time. The shaded 17° arc is necessary, since the hygrothermograph clocks rotate the drums only 343° in 7 days.

used. This chart is prepared by making a circle with radii marked at 6-hour intervals, computed on the basis that hygrothermograph clocks rotate the drums 343° in 7 days. The plates are superimposed on the chart and records of time of occurrence and duration of wet periods may be read directly.

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THE EFFECT OF MINERAL SALTS ON LOCAL-LESION FORMATION BY PHASEOLUS VULGARUS INOCULATED WITH TOBACCO MOSAIC VIRUS [1]

James D. Panzer [2]

SUMMARY

Few lesions developed when Pinto bean plants inoculated with a standard inoculum of tobacco mosaic virus (TMV) were exposed only during the period of germination to a complete Meyer's solution or to such solutions deficient in nitrogen, phosphorus, or potassium. When the plants were maintained continuously in the solutions, numerous lesions developed, the greatest number occurring on plants in the complete solution, followed in turn by solutions deficient in K, P, and N. When the solutions were prepared with tap water, essentially the same trends were present as when distilled water was used, with the exception that the greatest number of lesions developed on the plants maintained in the solution deficient in phosphorus. Fewer lesions developed in this series than in the distilled water series. No lesions occurred on plants maintained continuously in single-salt solutions of N, P, K, or Fe or in a micrometabolic element solution. When bean plants were inoculated with TMV and placed in a complete Meyer's solution prepared with different proportions of distilled and tap water, the number of lesions decreased as the proportion of tap water increased. This same relationship was observed when different proportions of tap and demineralized water were used. In both cases, the amount of a precipitate in the bottom of the bottles increased as the amount of tap water increased. The pH of the complete Meyer's solution had little direct effect, if any, on lesion number, but the number was affected indirectly by the precipitation of the mineral salts. In tests with a cation series, greatest lesion numbers occurred in the area of high Mg^{++} with low K^{+} and Ca^{++} and in the area of high Ca^{++} and low Mg^{++} and K^{+}. In tests with an anion series, the greatest number of lesions occurred in the area of high $PO_4^{=}$ with low NO_3^{-} and $SO_4^{=}$.

Since local-lesion hosts such as *Nicotiana glutinosa* L. and *Phaseolus vulgarus* L. are used extensively in work with virus diseases of plants, a large amount of work on the physiology of these host plants *per se* is necessary. A knowledge of the effects of nutrition of such plants on the number of virus lesions produced on them would contribute to the understanding of host-virus relationships.

Chessin (3) found fewer lesions on calcium-deficient plants of *N. glutinosa* than on plants maintained in balanced nutrient solutions when such plants were inoculated with tobacco mosaic-virus (TMV). Sulfur deficiency had no significant effect on lesion development. Samuel and Bald (7) reported that frequent watering of plants of *N. glutinosa* resulted in "hard" plants that had fewer lesions when inoculated with TMV or with tomato spotted wilt virus than did "average" or "good" plants that received normal watering. They attributed the small number of lesions to the leaching of the minerals from the soil. In work with potassium nutrition, Allington and Laird (1) found that low potassium nutrition increased the tendency for plants of *N. glutinosa* to become infected with TMV. According to Bawden and Kassanis (2), additions of nitrogen or of potassium to plants of *N. glutinosa* increased the number of local lesions produced when such plants were inoculated with TMV or with tomato aucuba mosaic, provided that these minerals also increased growth.

The objective of this work was to determine the effect of mineral salt nutrition on the local-lesion host plant *P. vulgarus* as related to TMV infection. In all of these experiments, entire plants and not merely detached leaves were exposed to various nutrient solutions for various lengths of time after which the leaves of these plants were inoculated with TMV.

MATERIALS AND METHODS.—Seeds of the Pinto variety of the common bean *P. vulgarus* were sown in washed white sand in containers (Lily Nestrite Containers #27163) with small holes in the bottom. The containers were placed in 5-in. clay saucers. The sand was saturated with the test solution and each container covered with a clay saucer to prevent water loss (Fig. 1, A). Four- to 5-in. seedlings developed inside the covered containers in about 6 days. The roots of these seedlings were washed free of sand and placed in solutions contained in Mason jars that had been painted black on the outside to prevent algal growth. Plants were supported by holders consisting of 4 × ¼ × 16-in. pieces of wood with four ¼-in. slots at 4-in. intervals (the slots extended from the long edge of the board to the center). Two ¾-in. brads were placed on each side of the slots, on both the top and bottom of the boards, just outside the jars. The plants were held in place by 2 thin rubber bands stretched between the brads on the top and on the bottom of the board (Fig. 1, B).

Because of high plant survival and ease of operation, this method is considered superior to a number

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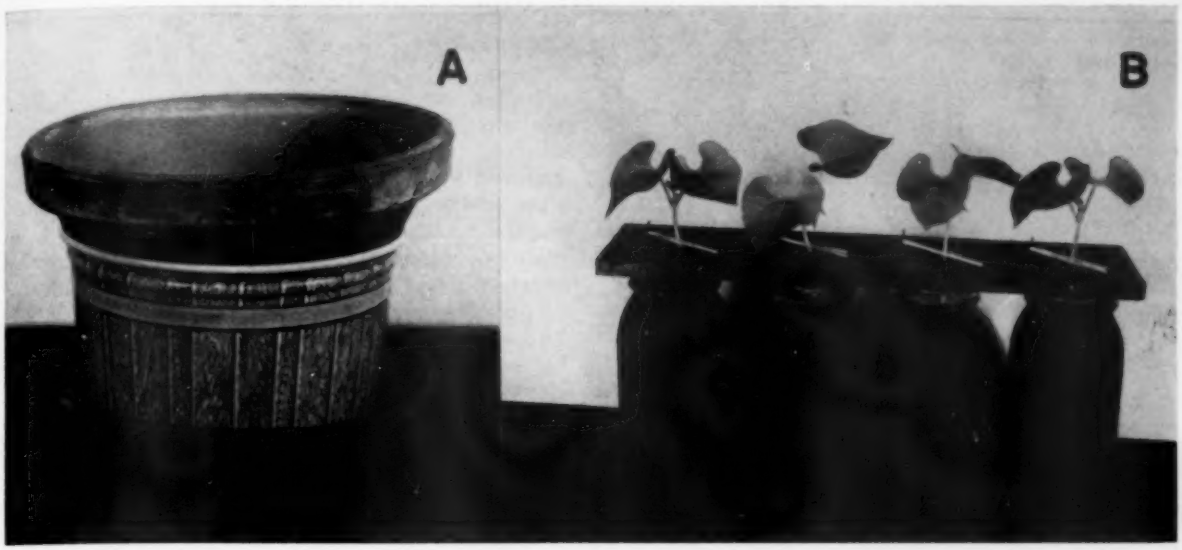

FIG. 1. Solution-culture apparatus. A) Containers in which seeds germinated. B) Mason jars with plant holders.

of methods commonly employed in solution culture of plants.

As a standard method of inoculation, the primary unifoliate leaves were dusted with 600-mesh Carborundum and then swabbed with a small piece of cheesecloth that had been dipped in the inoculum, which consisted of a mixture of 1 ml of a purified virus preparation suspended in 1000 ml of a 0.1 per cent K_2HPO_4 solution. When this dilution was used, fewer lesions occurred than usually result from inoculations made with undiluted or moderately dilute crude plant sap from diseased plants, but in the opinion of the author this method was as satisfactory and more consistent than the crude-sap method.

Since single solutions and single plants were placed in the Mason jars, and since the plants were treated and the leaves merely inoculated, treatments could be randomized by means of the Latin square method. In most experiments 5 × 5 Latin squares were used, with each square replicated. The data were analyzed by means of an analysis of variance. For the isolation of comparison with a single degree of freedom, an orthogonal comparison (4) was used.

RESULTS.—*Lesion development on plants grown in water, in a complete nutrient solution, and in nutrient solutions minus N, P, or K.*—Pinto bean seeds were placed in sand to which had been added either distilled water, a complete Meyer's solution (5), or Meyer's solution without N, P, or K. The formulae are given in Table 1. Six days later, the seedlings were removed and placed in distilled water in the Mason jars. Another group of seeds was treated in the same manner except that the seedlings were placed in the jars in the same solutions in which the seeds had germinated. A similar series of plants were prepared, with the exception that the water used in the preparation of the solutions was tap and not distilled water. All plants were inoculated with TMV 7 days after they had been placed in the Mason jars.

In the distilled water series, no lesions occurred on the plants that had received mineral salts only during germination (Table 2). Lesions developed on all of the plants maintained continuously on mineral salts; the differences between the number of lesions on the check plants (water) and that on plants on all treatments except −N were statistically significant.

Mineral salts appeared to be related to lesion formation, a balanced solution resulting in the greatest number. Nitrogen appeared to have the greatest effect, followed in order by phosphorus and potassium.

TABLE 1.—*Composition of the complete and modified Meyer's solution used*

Solution[a]	No. of ml of 1 M solutions of indicated salts used for each ml of test solution[b]							
	KNO_3	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	NaH_2PO_4	$NaNO_3$	KCl	$CaCl_2$
Complete	2	2	3	2				
Minus K			3	2	2	2		
Minus N		2		2			2	3
Minus P	2		3	2			2	

[a] To each liter of the respective solutions, 1 ml of a ferric tartrate solution and 1 ml of a micrometabolic element solution were added (2).

[b] The calculated osmotic pressures of all of the solutions lie within the range of 0.50 ± 0.05 atmospheres, and their initial pH values were 4.0 ± 0.2.

TABLE 2.—*Lesion development following the inoculation with tobacco mosaic virus of Pinto bean leaves on plants that were maintained in various nutrient solutions for different periods*

Solution composition	Average no. of lesions per leaf[a] on plants treated during indicated period with solutions prepared with distilled water or with tap water			
	Distilled water		Tap water	
	During[b] germination	Continuously	During[b] germination	Continuously
Water	0	0	0.3	0.5
Complete[c]	0	69	0.3	3.0
—N	0	5	0.3	0.8
—P	0	18	0.3	4.0
—K	0	24	0.3	0.3

[a] Average of 10 leaves per treatment. For distilled water series, $F = 20.0^{**}$ (5%, 2.58; 1%, 3.78); for tap water series, $F = 12.71^{**}$ (5%, 2.63; 1%, 3.89).
[b] After germination, seedlings were supplied water only.
[c] Meyer's solution (5).

In the tap water series, a few lesions developed when the plants were subjected to the mineral salts only during germination. With respect to the plants continuously supplied with mineral salts, there were statistically significant differences between the number of lesions on those given the complete-salt or —P treatments and the number on the check plants (water) and between the number on plants given the —N and —K treatments and the number on plants given the complete-salt treatment. Essentially the same trends were present as when distilled water was used, except that the greatest number of lesions developed on plants maintained in the solution deficient in phosphorus. In this series, fewer lesions were noted than in the distilled water series.

Effect of water source.—Complete Meyer's solutions with various amounts of tap and demineralized water were used in an experiment on the effect of water source on lesion number. Pinto bean plants were supplied with the solutions throughout the experiment. The percentage of the water that was tap water in the 5 solutions used was 0, 25, 50, 75, and 100. Another series was prepared with tap and demineralized water with percentages of tap water of 0, 25, 50, and 100. Plants were inoculated 7 days after being placed in Mason jars holding the solutions.

In both the distilled water and the demineralized water series, there was a negative association of the mean of the number of lesions per leaf with the amount of tap water in the solution, i.e. the greater the proportion of tap water, the smaller the number of lesions (Table 3). The amount of precipitate found in the bottom of the jars increased as the amount of tap water increased. Apparently some of the minerals in the Meyer's solutions had precipitated.

Since the tap water used was slightly basic and the distilled and demineralized water were slightly acid, the precipitation of the minerals could be related to the pH of the solutions. Also, pH might have had an effect on lesion number.

Effect of pH.—A complete Meyer's solution was prepared with tap water and another with distilled water. The pH values of the 2 solutions were 6.0 and 4.8, respectively. An additional Meyer's solution was prepared with tap water and adjusted to pH 4.8; another was prepared with distilled water and adjusted to pH 6.0. When bean plants grown on these 4 solutions were inoculated with TMV, the mean number of lesions per leaf were as follows: tap water, pH 6.0—0.9; distilled water, pH 4.8—3.9; tap water, pH 4.8—3.1; and distilled water, pH 6.0—4.0. The difference between the mean for the tap water solution (pH 6) and that for each of the other treatments was statistically significant. Very little precipitate occurred in the jars, with the exception of the one containing Meyer's solution prepared with tap water and not adjusted.

The pH of the solution apparently affected lesion number indirectly by precipitation of the minerals in the Meyer's solution. That the pH had no direct effect on lesion number is evidenced by the fact that there were no statistically significant differences between the means for the 2 solutions prepared with distilled water but differing in pH.

Effect of single salts.—Bean plants were placed in Mason jars containing 1) distilled water; 2) complete Meyer's solution; 3) 5 ml 1 M $Ca(NO_3)_2$ per liter; 4) 2 ml 1 M NaH_2PO_4 per liter; 5) 2 ml 1 M KCl per liter; 6) 1 ml of iron tartrate solution per liter (5); or 7) 1 ml of micrometabolic element solution (5) per liter. After the plants had been maintained in these solutions for 7 days, they were inoculated with TMV.

Lesions developed only on the plants that had received the complete Meyer's solution. The Fe and K solutions were phytotoxic. Thus, although lesions de-

TABLE 3.—*Lesion formation on Pinto bean plants maintained for 4 days on Meyer's solution prepared with various proportions of tap water, distilled water, and/or demineralized water and then inoculated with tobacco mosaic virus*

Percentage tap water[b]	Mean no. of lesions[a] per leaf when remainder of water was as indicated	
	Distilled water	Demineralized water
0	15.2	6.6
25	8.7	2.9
50	4.5	1.4
75	—[c]	1.0
100	2.6	0.5

[a] In both series, the amount of precipitate that formed in Meyer's solution with 0, 25, 50, 75, and 100 per cent tap water was rated none, slight, medium, medium, and heavy, respectively.
[b] Average of 10 plants (20 leaves) per treatment. Regression coefficients for the regression of the mean number of lesions on increasing amounts of tap water were —4.2 (S.D. = 0.78) for the distilled water and —1.41 (S.D. = 0.35) for the demineralized water.
[c] No data available.

TABLE 4.—*Mean number of lesions per leaf on Pinto bean leaves on plants maintained in nutrient solutions containing different proportions of certain cations or anions and inoculated with tobacco mosaic virus*

Relative proportions of cations or anions[a]	Mean no. of lesions/leaf[b]	
	Cation series	Anion series
4-1-1	2	2
3-2-1	1	3
3-1-2	0	1
2-3-1	7	3
2-2-2	6	3
2-1-3	3	3
1-4-1	4	3
1-3-2	5	3
1-2-3	11	3
1-1-4	2	2

[a] In cation series, proportions of cations in the order K^+, Mg^{++}, Ca^{++}. In the anion series, the order is NO_3^-, $PO_4^=$, $SO_4^=$.

[b] Mean of 10 leaves per treatment. For cation series, $F = 2.32^*$ (5%, 1.94). For anion series, $F = 2.00^*$ (5%, 1.94).

veloped on plants grown in Meyer's solution, none developed on plants grown on solutions of the component salts used singly.

Cation series.—A cation series (6) consisting of solutions of different proportions of K^+, Ca^{++}, and Mg^{++}, was prepared. These solutions were such that the amount of anions remained constant whereas the proportions of the 3 cations were varied.

Greatest lesion numbers occurred in the area of high Mg^{++} with low K^+ and Ca^{++} and in the area of high Ca^{++} and low Mg^{++} and K^+ (Table 4).

Anion series.—An anion (6) series, in which the amount of cations in the solutions remained constant whereas the proportions of the anions NO_3^-, $PO_4^=$, and $SO_4^=$ were varied, was prepared. Greatest lesion numbers occurred in the area of high $PO_4^=$ with low NO_3^- and $SO_4^=$ (Table 4).

DISCUSSION.—Obviously the number of local lesions that develop on leaves of Pinto bean plants is affected by the mineral salts supplied previous to and following inoculation with TMV. A deficiency of any of the major nutritive elements reduces susceptibility. This reduction in susceptibility might be effected if test plants grew in soil that was deficient in certain minerals or if the plants were watered with a type of water that would cause the precipitation of certain of the mineral salts in the soil.

Even if the soils used are not deficient in any mineral salts, variability in plant susceptibility may be introduced by any alteration in the relative proportions of the mineral salts. Thus the lack of a uniform application of fertilizer could be a factor in the variability in plant response to inoculation with a local-lesion-inducing virus.

Even if extreme care were used as to water source, soil uniformity, and the type and amount of fertilizer supplied to the test plants, the amount of water used could affect lesion number because of leaching effects as reported by Samuel and Bald (7). For quantitative work with local-lesion hosts, it is suggested that a solution culture be used to minimize the effects of mineral salt "nutritional" variation. If soil-grown plants are used, standardization of soil, the amount and source of water, and the type and amount of fertilizer supplied to the test plants is necessary.

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A PHYTOPATHOLOGICAL NOTE

A Flotation Method for Collecting Spores of a Phycomycetous Mycorrhizal Parasite from Soil. RICHARD E. OHMS. The only source of the spores of an apparently obligate, phycomycetous mycorrhizal parasite that has been reported in South Dakota[1] and elsewhere[2] is naturally infested soil. For inoculation purposes, Gerdemann[2] handpicked spores from an aqueous suspension of soil screenings retained by a No. 60 sieve with 0.25-mm openings. The procedure proved time consuming and yielded about 100 spores

[1] Ohms, R. E. 1956. A phycomycetous mycorrhiza on barley roots in South Dakota. Plant Disease Reptr. 40: 507.

[2] Gerdemann, J. W. 1955. Relation of a large soil-borne spore to phycomycetous mycorrhizal infections. Mycologia 47: 619-632.

per man day. A better method was needed to provide a greater number of spores for inoculation purposes. Accordingly, a flotation technique was investigated after the density of the spores in the water was observed to be intermediate between that of the heavier soil and that of the lighter plant debris. The following procedure was developed and yielded more spores than that used by Gerdemann.[2]

Soils were screened essentially according to Gerdemann's method[2] to yield a fraction retained by a No. 80 sieve with 0.177-mm openings. The screenings were suspended in water and processed in 2 steps. First, the light material was decanted after the spores and heavier soil particles had settled in standing beakers or had been centrifuged for 3 minutes at 3100 rpm. Second, a suspension of the spores and remaining soil debris was added to a 50-ml centrifuge tube containing 50 per cent sucrose at the bottom (10 ml), a middle layer of 25 per cent sucrose (15 ml), and a top layer of water (10 ml). The middle and top layers had been carefully placed into position by means of a hypodermic syringe supplied with a curved glass tube extension to direct the liquid against the side of the vertically held tube. The top layer of water served to disperse the screenings and thus to reduce the number of spores that otherwise would be carried into the bottom layer. After a 5-minute centrifuging at 3100 rpm, the spores had settled into the middle layer whereas the soil particles had settled into the bottom layer.

The spores from the middle layer, along with some of the 25 per cent solution, were removed with a pipette and transferred to a beaker. Water was next added to this suspension to decrease the specific gravity of the solution and to allow the spores to settle. The solution was then decanted and the spores washed several times with water. The spores were then ready for hand sorting and could then be freed of remaining debris. The method yielded 2350 spores in 1.5 days.—Department of Plant Pathology, South Dakota Agricultural Experiment Station, Brookings, South Dakota.

LISTING OF BOOKS

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on pathological subjects. A brief description of the contents of the books will be included.

BECKER, G., AND GERDA THEDEN, ed. **Annual report on wood protection 1955.** Springer, Berlin, Göttingen, Heidelberg.

This digest of world literature on the protection of wood against deterioration was founded by Willy Kinberg about 20 years ago and has been continued by the present editors since 1951. As in previous issues, the literature abstracted in the 1955 edition is confined to publications that present new data of value in developing and applying protective measures. Most of these publications appeared in 1955 or early 1956; the remainder represent previously overlooked papers from the 1952–1954 period. Beginning with this edition, the Report is expected to be released annually again.

In general content, the new edition comprises about 300 pages divided into 4 sections—classification of subject matter, abstracts arranged according to this classification, bibliography, and index. The abstracts are presented under the following headings: Fundamentals of Wood Protection; Wood-Destroying Organisms and Their Influence; Wood Protection Against Organisms; Fire-Retarding Wood Protection; Wood Protection Against Chemical Agents; Economical Aspects and Statistics; Reports on Scientific Institutes; Associations, Conventions, International Cooperation; and Personal Notes.

The abstracts, which range from 20 to 200 or more words, are given in both German and English, on facing pages. They carry the author's name and are numbered consecutively. A new feature is the inclusion of marginal notes to facilitate the search for abstracts of special interest within the broad subject groupings. Also, literature on marine organisms in wood is covered intensively for the first time.

DOWSON, W. J. 1957. **Plant diseases due to bacteria.** Cambridge University Press, New York. 232 p. Price $6.50.

This is a second edition of the author's *Manual of bacterial plant diseases* (1949). The present edition follows the plan of the earlier work, with the addition of considerable new material and a chapter on preservation of cultures. A general discussion of bacteriological techniques and of classification and nomenclature of the bacteria is followed by an account of the more important bacterial plant diseases. Many are illustrated by half-tones and their world distribution indicated by maps.

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NEWS

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

The AAAS-Campbell Award for vegetable research has just been established by the Campbell Soup Company and will be given for the first time in 1957. The award consists of $1500 and a bronze medal, given for an outstanding single research contribution, of either fundamental or practical significance, relative to the production of vegetables including mushrooms, for processing purposes in the fields of horticulture, genetics, soil science, plant physiology, entomology, plant pathology or other appropriate scientific areas. The one or more papers reporting this research should have been published—or the manuscript accepted for publication—in a recognized scientific journal, not more than two years prior to the date of granting the award, which is open to all residents of the U. S. and Canada. Travel expenses for the recipient, to attend the AAAS meetings and to receive the award in person, are paid in addition. The Award Committee is composed of a chairman appointed by the AAAS and official representatives of six affiliated societies.

Plant pathologist C. E. Yarwood, University of California, Berkeley, is on sabbatical leave at the Virus Research Unit, Molteno Institute, Cambridge, England, as a Guggenheim Fellow.

Witchweed will be the subject of an invitational paper to be presented by phytopathologist R. R. Nelson at the 12th annual Hybrid Corn Industry Research Conference in Chicago early in December.

Phytopathologist Harold T. Cook has been appointed assistant chief of the Biological Sciences Branch, Marketing Research Division, U.S.D.A., succeeding Dr. Charles E. Sando who retired.

Phytopathologist John M. Harvey has been named head of the U.S.D.A. Field Station, Fresno, California. Dr. Harvey has made significant contributions in the control of grape storage diseases.

Tobacco leaf diseases and their control in Panama will be investigated by G. B. Lucas, Associate Professor of Plant Pathology, North Carolina State College. Dr. Lucas will make trips to that area in November and January.

The Department of Botany and Plant Pathology at Oregon State College has moved to new and expanded quarters. It now occupies part of four floors in recently constructed Cordley Hall on the Corvallis campus.

Phytopathologist L. J. Klotz, recently on sabbatical leave in Europe and North Africa, has just returned to the Riverside campus, University of California. While on leave he visited laboratories and studied citrus diseases and other plant disease problems as they occur in Spain, Portugal, Italy, Sicily, Morocco, Canary Islands and France. During his travels he presented a paper at the IV International Congress of Plant Protection in Hamburg, Germany and also visited laboratories in Germany, Switzerland and the Netherlands.

The Potomac Division will hold its fifteenth annual meeting in the auditorium, Plant Industry Station, Beltsville, Maryland, on February 27 and 28.

Deaths—John W. Roberts, retired, former Principal Pathologist in charge of Fruit Disease Investigations, U.S.D.A., was killed along with Mrs. Roberts in an automobile accident near Winter Park, Florida. Dr. Roberts was a Charter and Life Member of the Society. He retired in 1945 after 36 years in government service. Dr. Roberts conducted early research on apple leafspots and the apple bitter rot fungus.

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